(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 February 2008 (21.02.2008)

PCT

(10) International Publication Number
WO 2008/021439 A2

(51) International Patent Classification:
A61K 31/675 (2006.01) *A61K 31/554* (2006.01)

(21) International Application Number: PCT/US2007/018147

(22) International Filing Date: 15 August 2007 (15.08.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PCT/US2006/32405	17 August 2006 (17.08.2006)	US
11/506,285	17 August 2006 (17.08.2006)	US
60/904,349	28 February 2007 (28.02.2007)	US
11/809,470	1 June 2007 (01.06.2007)	US

(71) Applicant (*for all designated States except US*): **THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK** [US/US]; 412 Low Memorial Library, 535 West 116th Street, New York, NY 10027 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **MARKS, Andrew, Robert** [US/US]; 12 Locust Avenue, Larchmont, NY 10538 (US). **LEHNART, Stephan, E.** [DE/US]; 324 West 113 Street, Apartment 1A, New York, NY 10026 (US).

(74) Agents: **LOVE, Jane, M.** et al.; Wilmer Cutler Pickering Hale And Dorr LLP, 399 Park Avenue, New York, NY 10022 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2008/021439 A2

(54) **Title:** COMPOSITIONS AND METHODS FOR TREATMENT OF CARDIAC HYPERTROPHY

(57) **Abstract:** The present invention provides methods and compositions useful for the treatment and/or prevention of cardiac hypertrophy. In certain embodiments, the methods of the present invention comprise administering to a subject a therapeutically or prophylactically effective amount of a 1,4, benzothiazepine compound.

COMPOSITIONS AND METHODS FOR TREATMENT OF CARDIAC HYPERTROPHY

[0001] This application claims the benefit of priority of International Application PCT/US2006/32405 filed August 17, 2006; this application also claims the benefit of priority of U.S. Application No: 11/506,285 filed August 17, 2006, this application also claims the benefit of priority of U.S. Application No: 60/904,349 filed February 28, 2007; this application also claims the benefit of priority of U.S. Application No 11/809,470 filed June 1, 2007, which is a continuation-in-part of International Application PCT/US2006/032405 filed August 17, 2006. The disclosures of these applications in their entirety are hereby incorporated by reference into this application. Furthermore, the text of all other patent applications, published patents applications, issued and granted patents, and all references cited in this application, are hereby incorporated by reference in their entirety. For example, the contents of U.S. patent applications 09/568,474, 10/288,606, 10/680,988, 10/608,723, 10/809,089, 10/763,498, 10/794,218, 11/088,058, 11/088,123, 11/212,309, and 11/212,413, are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates to methods and compositions useful for the treatment and/or prevention of cardiac hypertrophy.

BACKGROUND OF THE INVENTION

[0003] Cardiac hypertrophy is a condition of the heart characterized by an increase in size of the cardiac muscle. In some situations cardiac hypertrophy occurs as an adaptation of the heart to increased demand on cardiac output, for example as a result of hypertension, aortic stenosis, myocardial infarction, congestive heart failure, and other pathologic conditions associated with chronic hemodynamic overload. Cardiac hypertrophy can also be caused by genetic abnormalities. For example, hypertrophic cardiomyopathies are genetic disorders which may manifest as cardiac hypertrophy in the absence of chronic hemodynamic overload. Studies have suggested that the prevalence of hypertrophic cardiomyopathies may be as high as 1 in 500 in the population. Cardiac hypertrophy is a significant risk factor for both mortality and morbidity in heart failure patients, and, in the case of hypertrophic cardiomyopathy, is also a leading cause of unexpected sudden cardiac death in seemingly healthy individuals.

[0004] Sudden cardiac death (SCD) due to ventricular arrhythmias is a leading cause of mortality in patients with heart disease. The mechanisms that trigger fatal arrhythmias are incompletely understood and treatment and prevention remain largely ineffective. Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is characterized by autosomal-dominant inheritance of syncope, tachyarrhythmias, or SCD associated with multifocal ventricular tachycardias during physical or emotional stress but not during rest (Leenhardt et al., 1995). CPVT was linked to missense mutations in the cardiac ryanodine receptor gene RyR2 (Laitinen et al., 2001; Priori et al., 2001). RyR2 mutations were also reported in patients with CPVT and additional fibro-fatty right ventricular changes which apparently do not fulfill the task force criteria for Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) (Tiso et al., 2001).

[0005] There is therefore a need in the art for new and improved methods of prevention, treatment, and diagnosis of cardiac hypertrophy. The present invention addresses these and other needs in the art by providing, *inter alia*, compositions and methods useful for the treatment of cardiac hypertrophies. These compositions and methods involve modulation of the function of cardiac ryanodine receptors.

[0006] Ryanodine receptors (RyRs) are channels in the sarcoplasmic reticulum (SR) that open and close to regulate the release of Ca^{2+} from the SR into the intracellular cytoplasm of the cell. The "open probability" (Po) of a RyR receptor refers to the likelihood that the RyR channel is open at any given moment, and therefore capable of releasing Ca^{2+} into the cytoplasm from the SR.

[0007] There are three types of ryanodine receptors, all of which are Ca^{2+} channels: RyR1, RyR2, and RyR3. RyR1 is found predominantly in skeletal muscle as well as other tissues, RyR2 is found predominantly in the heart as well as other tissues, and RyR3 is found in the brain as well as other tissues. The RyR channels are formed by four RyR polypeptides in association with four FK506 binding proteins (FKBPs), specifically FKBP12 (calstabin1) and FKBP12.6 (calstabin2). Calstabin1 binds to RyR1, calstabin2 binds to RyR2, and calstabin1 binds to RyR3. The FKBP proteins (calstabin1 and calstabin2) bind to the RyR channel (one molecule per RyR subunit), stabilize RyR-channel functioning, and facilitate coupled gating between neighboring RyR channels, thereby preventing abnormal activation of the channel during the channel's closed state.

[0008] The function of RyRs is also regulated by phosphorylation. PKA phosphorylation of RyRs causes partial dissociation of calstabins from RyRs. Dissociation of calstabin from RyR increases the open probability of RyRs, and thereby increased Ca^{2+} release from the SR into the cytoplasm.

SUMMARY OF THE INVENTION

[0009] There is a need in the art for new agents and methods for treating, preventing, and diagnosing cardiac hypertrophy. The present invention is based, in part, on the discovery that mutations in the RyR2 channel in individuals with hypertrophic cardiomyopathy result in an increased open probability of the RyR2 channel or a "leakiness" of the RyR2 channel, and that this appears to be a causative factor in the development of cardiac hypertophy. In certain embodiments, the present invention provides methods and compositions for the treatment, prevention and/or amelioration of cardiac hypertrophy. These and other aspects of the invention are described in the below summary, the detailed description, the examples, and the claims.

[0010] In one embodiment, the present invention provides a method of treating, preventing, or ameleorating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound of Formula I, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites, or pro-drugs thereof, or any combination thereof. The structure of Formula I is provided in the below Detailed Description.

[0011] In another embodiment, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound represented by the structure of Formula I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites, or pro-drugs thereof, or any combination thereof. The structure of Formulae I-a to I-p and Formula II are provided in the below detailed description.

[0012] In another preferred embodiment, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to

the subject a therapeutically or prophylactically effective amount of a compound represented by the structure of Formula I-a, I-b, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-n, I-o, or I-p, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites, or pro-drugs thereof, or any combination thereof.

[0013] In certain preferred embodiments, the compound administered is selected from the group consisting of S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123. The structures of these compounds are provided in the below detailed description.

[0014] In further preferred embodiments, the compound administered is selected from the group consisting of S36, S47, S50, S64, S74, S75, S77, S101, S102, S103, S017, S110, S111, and S117.

[0015] In further preferred embodiments, the compound administered is selected from the group consisting of S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123.

[0016] In further preferred embodiments, the compound administered is selected from the group consisting of S101, S102, S103, S107, S110, S111, and S117.

[0017] In particularly preferred embodiments, the compound administered is S36. In other particularly preferred embodiments the compound is S64.

[0018] In other embodiments, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases the open probability of the RyR2 channel.

[0019] In yet another embodiment, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the

subject a therapeutically or prophylactically effective amount of a compound that decreases Ca2+ current through the RyR2 channel.

[0020] In a further embodiment, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases calcium leak through the RyR2 channel.

[0021] In an additional embodiment, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases the affinity with which calstabin 2 binds to RyR2.

[0022] In other embodiments, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases dissociation of calstabin 2 from RyR2.

[0023] In other embodiments, the present invention provides a method of treating or preventing cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases rebinding of calstabin 2 to RyR2.

[0024] In certain embodiments, the subject to whom the compounds of the invention are administered is a mammal selected from the group consisting of primates, rodents, ovine species, bovine species, porcine species, equine species, feline species and canine species. In a preferred embodiment, the subject is a human.

[0025] The subjects of the invention may be suffering from hemodynamic overload. In certain preferred embodiments, the subject is suffering from hypertension, aortic stenosis, myocardial infarction, congestive heart failure, idiopathic hypertrophic subaortic stenosis (IHSS), hypertrophic obstructive cardiomyopathy (HOCM), apical hypertrophic cardiomyopathy, non-obstructive hypertrophic cardiomyopathy, or some other type of chronic hemodynamic overload, sudden cardiac death, syncopal events, or a cardiac arrhythmia, such as ventricular fibrillation, ventricular tachycardia, bradycardia, long QT syndrome, QT 455ms, or an exercise induced arrhythmia. The subjects of the invention may

also be suffering from various structural and/or functional cardiac abnormalities, including, but not limited to, ASH (assymertic septal hypertrophy); LVOTO (left ventricular outflow tract obstruction); and DCO (distal cavity obliteration).

[0026] The subjects of the invention may alternatively or additionally have a mutation that is associated with development of cardiac hypertrophy. Such a mutation may be an inherited mutation or a sporadic mutation. In one preferred embodiment, the subject has a mutation in the gene that encodes RyR2.

[0027] In yet another preferred embodiment, the subject has a mutation that results in increased open probability of the RyR2 channel, or that results in increased Ca2+ current through the RyR2 channel, or that results in calcium leak through the RyR2 channel, or that decreases the affinity with which calstabin 2 binds to RyR2, or that increases dissociation of calstabin2 from RyR2, or that decreases rebinding of calstabin 2 to RyR2.

[0028] In a particularly preferred embodiment, the subject has a mutation selected from the group consisting of the R929C mutation, the G2367 R mutation, the R2642K mutation and the E3654D mutation. These mutations are further described in the below detailed description.

[0029] The compounds of the invention may be administered by any suitable route known in the art, without limitation. For example, compounds of the invention may be administered by a route selected from the group consisting of parenteral, enteral, intravenous, intraarterial, intracardiac, intra intrapericardial, intraosseal, intracutaneous, subcutaneous, intradermal, subdermal, transdermal, intrathecal, intramuscular, intraperitoneal, intrasternal, parenchymatous, oral, sublingual, buccal, rectal, vaginal, inhalational, and intranasal. Additionally, the compounds of the invention may be administered using a drug-releasing implant.

[0030] In one preferred embodiment, the compounds of the invention are administered to the subject at a dose sufficient to restore binding of calstabin2 to RyR2, or at a dose sufficient to enhance binding of calstabin2 to RyR2. In certain preferred embodiments, the compounds of the invention are administered to the subject a dose of from about 0.01 mg/kg/day to about 20 mg/kg/day, or more preferably still, at a dose of from about 0.05 mg/kg/day to about 1

mg/kg/day. Other suitable dose ranges are provided in the Detailed Description and Examples. In addition, one of skill in the art can select other suitable doses for administration.

[0031] Other features and advantages of the present invention will become apparent from the following description. It should also be understood that various changes and modifications to the methods and compositions described herein are possible without departing from the spirit and scope of the invention. Variations and modifications that can be made without departing from the spirit and scope of the invention will be apparent to those skilled in the art, and all such variations and modifications are within the scope of the invention. For example, further variations and modifications of the invention may be made in accordance with the description provided in U.S. patent applications 09/568,474, 10/288,606, 10/680,988, 10/608,723, 10/809,089, 10/763,498, 10/794,218, 11/088,058, 11/088,123, 11/212,309, 11/506,285, and 11/212,413, 60/904,349 and 11/809,470, and International application PCT/US2006/32405, the contents of which are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURES

[0032] Figure 1 provides immunoblotting data from microsomes containing WT or HCM mutant RyR2 in the absence (PKA+PKI) or presence (PKA) of PKA phosphorylation. The specific HCM mutations are indicated at the top of the lanes. The upper panel shows total RyR2 protein. The middle panel shows PKA phosphorylated RyR2-S2808 detected using a phosphospecific antibody (anti-RyR2-P2808) which specifically recognizes the phosphorylated protein. The lower panel shows calstabin2 in the RyR2 complex.

[0033] Figure 2 provides a graph showing calstabin2 (FKBP12.6) binding to microsomes in wild type and HCM-mutant RyR2 channels. Scatchard analysis of [^{35}S]-labeled calstabin2 binding to RyR2-WT, RyR2-R929C, RyR2-G2367R, RyR2-R2642K, and RyR2-E3654D channels is indicated.

[0034] Figure 3 provides single channel patch clamp data from wild type and HCM-mutant RyR2 channels. Panel (**A**) shows representative single-channel experiments from RyR2 channels treated with PKA and the PKA inhibitor PKI_{5-24}, representing the non-phosphorylated situation. Panel (**B**) shows representative single-channel experiments from RyR2 channels treated with PKA alone, representing the phosphorylated situation. In both Panels A and B channel openings are upward deflections, the difference between horizontal

bars indicates 4 pA level between open and closed state as indicated by the letter 'c'. Temporal resolution is 5000 ms for the1 upper and 500 ms for the lower traces where 1pA subconductance levels are indicated. Abbreviations used are: Po, open probability; To, average open time; Tc average closed time.

[0035] Figure 4 provides bar graphs showing open probability (Po) of unphosphorylated and PKA phosphorylated HCM-mutant, CPVT-mutant and WT RyR2 channels. Panel **(A)** provides data from non-phosphorylated RyR2 channels. Panel **(B)** provides data following PKA phosphorylation. Panel **(C)** contains a summary bar graph comparing the open probabilities in non-phosphorylated and PKA phosphorylated RyR2 channels. Asterisks (*) represent data points having a *P* value of < 0.05 as compared to WT RyR, and # symbols represent data points having a *P* value of < 0.05 as compared to the HCM mutant RyR2-G2367R.

DETAILED DESCRIPTION OF THE INVENTION

[0036] The following are definitions of terms used in the present specification. The initial definition provided for a chemical group or term herein applies to that group or term throughout the present specification individually or as part of another group, unless otherwise indicated.

[0037] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the content clearly dictates otherwise. Thus, for example, reference to "an agent" or "a compound" includes a plurality of such agents or compounds and equivalents thereof known to those skilled in the art.

[0038] As used herein, the term "cardiac hypertrophy" is used to refer to any disease, condition, or disorder characterized by enlargement or "hypertrophy" of the heart (for example by enlargement of the individual cardiac cells), regardless or etiology. Thus, as used herein, the term "cardiac hypertrophy" encompasses abnormal enlargement of the heart muscle caused by genetic mutations. Such mutations may be familial (i.e. inherited from a parent), or may be sporadically occurring (i.e. newly arising in an individual without a traceable hereditary path). Genetic disorders which result in cardiac hypertrophy are often referred to as hypertrophic cardiomyopathies.

[0039] The term “cardiac hypertrophy”, as used herein, also encompasses abnormal enlargement of the heart muscle not caused by a genetic mutation. Such “secondary” cardiac hypertrophy may occur in response to aortic stenosis, myocardial infarction, congestive heart failure, and other pathologic conditions associated with chronic hemodynamic overload. Other types of cardiac hypertrophy encompassed by the present invention or structural and/or functional abnormalities associated therewith, include, but are not limited to, assymetric septal hypertrophy (ASH), left ventricular outflow tract obstruction (LVOTO), and distal cavity oblitaration (DCO), idiopathic hypertrophic subaortic stenosis (IHSS), hypertrophic obstructive cardiomyopathy (HOCM), apical hypertrophic cardiomyopathy, and non-obstructive hypertrophic cardiomyopathy. Other conditions and diseases characterized by hypertrophy of the heart muscle will be known to those of skill in the art, and all such conditions and diseases are encompassed by the present invention.

[0040] The terms “mutation”, “mutant”, as used herein, refer to nucleic acid (such as DNA or mRNA) sequences or amino acid sequences that differ from “wild-type” nucleic acid or amino acid sequences in that they contain an insertion, deletion and/or substitution of one or more nucleotides or amino acids as compared to the corresponding “wild-type” nucleic acid or amino acid sequences, and to processes by which such modified nucleic acid or amino acid sequences arise or are generated. For example, in certain preferred embodiments, the term the term “mutant” or “mutation” is used to refer to a nucleic acid (such as DNA or mRNA) that encodes a RyR protein in which one or more amino acids is altered as compared to the corresponding “wild-type” RyR protein. In certain preferred embodiments, the mutation is one that alters the function of the RyR protein. In other preferred embodiments, the mutation is one that alters the function of a calstabin protein. In yet other preferred embodiments, the mutation is one that alters the interaction between a RyR protein and a calstabin protein. Further examples of mutations that are encompassed by the present invention are provided below.

[0041] As used herein, the term “misense mutation” refers to a mutation that results in the substitution of one amino acid for a different amino acid. Such a mutation may be a point mutation or a larger mutation. The substitution of the amino acid can be either "conservative amino acid substitution" or "non-conservative amino acid substitution". The term "conservative amino acid substitution" refers to an amino acid substitution which does not alter the relative charge or size characteristics of the polypeptide in which the amino acid

substitution is made. A conservative amino acid substitution typically creates a mutant polypeptide that is functionally equivalent to the wild type polypeptide. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) Methionine (M), Isoleucine (I), Leucine, Valine (V); (b) Phenylalanine (F), Tyrosine (Y), Tryptophane (W); (c) Lysine (K), Arginine (R) (d) Alanine (A), Gglycine (G); (e) Serine (S), Threonine (T); (f) Glutamine (Q), Asparagine (N); and (g) Glutamic acid (E), Aspartic acid (D). As used herein, the term "non-conservative amino acid substitution" refers to an amino acid substitution which is not a conservative amino substitution, and which results in an amino acid or polypeptide which is functionally different from the non-substituted amino acid or polypeptide.

[0042] As used herein, the term "RyCal compounds" refers to compounds of the general Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, as provided by the invention, and herein referred to as "compound(s) of the invention".

[0043] The compounds of the invention are referred using a numerical naming system, with compound numbers 1 to 123 provided herein. These numbered compounds are referred to using either the prefix "S" or the prefix "ARM." Thus, the first numbered compound is referred to either as "S1" or "ARM001", the second numbered compound is referred to as either "S2" or "ARM002", the third numbered compound is referred to as either "S3" or "ARM003", and so on. The "S" and the "ARM" nomenclature systems are used interchangeably throughout the specification, the drawings, and the claims.

[0044] The term "alkyl" as used herein refers to a linear or branched, saturated hydrocarbon having from 1 to 6 carbon atoms. Representative alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, isohexyl, and neohexyl. The term "C_1-C_4 alkyl" refers to a straight or branched chain alkane (hydrocarbon) radical containing from 1 to 4 carbon atoms, such as methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl, and isobutyl.

[0045] The term "alkenyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon double bond. In one embodiment, the alkenyl has one or two double bonds. The alkenyl moiety may exist in the E or Z conformation and the compounds of the present invention include both conformations.

[0046] The term "alkynyl" as used herein refers to a linear or branched hydrocarbon having from 2 to 6 carbon atoms and having at least one carbon-carbon triple bond.

[0047] The term "aryl" as used herein refers to an aromatic group containing 1 to 3 aromatic rings, either fused or linked.

[0048] The term "cyclic group" as used herein includes a cycloalkyl group and a heterocyclic group.

[0049] The term "cycloalkyl group" as used herein refers to a three- to seven-membered saturated or partially unsaturated carbon ring. Any suitable ring position of the cycloalkyl group may be covalently linked to the defined chemical structure. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

[0050] The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

[0051] The term "heterocyclic group" or "heterocyclic" or "heterocyclyl" or "heterocyclo" as used herein refers to fully saturated, or partially or fully unsaturated, including aromatic (i.e., "heteroaryl") cyclic groups (for example, 4 to 7 membered monocyclic, 7 to 11 membered bicyclic, or 10 to 16 membered tricyclic ring systems) which have at least one heteroatom in at least one carbon atom-containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, 3, or 4 heteroatoms selected from nitrogen atoms, oxygen atoms and/or sulfur atoms, where the nitrogen and sulfur heteroatoms may optionally be oxidized and the nitrogen heteroatoms may optionally be quaternized. The heterocyclic group may be attached to the remainder of the molecule at any heteroatom or carbon atom of the ring or ring system. Exemplary heterocyclic groups include, but are not limited to, azepanyl, azetidinyl, aziridinyl, dioxolanyl, furanyl, furazanyl, homo piperazinyl, imidazolidinyl, imidazolinyl, isothiazolyl, isoxazolyl, morpholinyl, oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, piperazinyl, piperidinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazolyl, pyridoimidazolyl, pyridothiazolyl, pyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, quinuclidinyl, tetrahydrofuranyl, thiadiazinyl, thiadiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiomorpholinyl, thiophenyl, triazinyl, and triazolyl. Exemplary bicyclic heterocyclic groups include indolyl, isoindolyl, benzothiazolyl, benzoxazolyl, benzoxadiazolyl, benzothienyl, quinuclidinyl, quinolinyl,

tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indolizinyl, benzofuryl, benzofurazanyl, chromonyl, coumarinyl, benzopyranyl, cinnolinyl, quinoxalinyl, indazolyl, pyrrolopyridyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl] or furo[2,3-b]pyridinyl), dihydroisoindolyl, dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl), triazinylazepinyl, tetrahydroquinolinyl and the like. Exemplary tricyclic heterocyclic groups include carbazolyl, benzidolyl, phenanthrolinyl, acridinyl, phenanthridinyl, xanthenyl and the like.

[0052] The term "phenyl" as used herein refers to a substituted or unsubstituted phenyl group.

[0053] The aforementioned terms "alkyl," "alkenyl," "alkynyl," "aryl," "acyl," "phenyl," "cyclic group," "cycloalkyl," "heterocyclyl," "heterocyclo," and "heterocycle" may further be optionally substituted with one or more substituents. Exemplary substituents include but are not limited to one or more of the following groups: hydrogen, halogen, CF_3, OCF_3, cyano, nitro, N_3, oxo, cycloalkyl, alkenyl, alkynyl, heterocycle, aryl, alkylaryl, heteroaryl, OR_a, SR_a, $S(=O)R_e$, $S(=O)_2R_e$, $P(=O)_2R_e$, $S(=O)_2OR_a$, $P(=O)_2OR_a$, NR_bR_c, $NR_bS(=O)_2R_e$, $NR_bP(=O)_2R_e$, $S(=O)_2NR_bR_c$, $P(=O)_2NR_bR_c$, $C(=O)OR_a$, $C(=O)R_a$, $C(=O)NR_bR_c$, $OC(=O)R_a$, $OC(=O)NR_bR_c$, $NR_bC(=O)OR_a$, $NR_dC(=O)NR_bR_c$, $NR_dS(=O)_2NR_bR_c$, $NR_dP(=O)_2NR_bR_c$, $NR_bC(=O)R_a$, or $NR_bP(=O)_2R_e$, wherein R_a is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl; R_b, R_c and R_d are independently hydrogen, alkyl, cycloalkyl, alkylaryl, heteroaryl, heterocycle, aryl, or said R_b and R_c together with the N to which they are bonded optionally form a heterocycle; and R_e is alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, alkylaryl, heteroaryl, heterocycle, or aryl. In the aforementioned exemplary substitutents, groups such as alkyl, cycloalkyl, alkenyl, alkynyl, cycloalkenyl, alkylaryl, heteroaryl, heterocycle and aryl can themselves be optionally substituted.

[0054] Exemplary substituents may further optionally include at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric or a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof. For example, ARM118 of the present invention contains a labeling group BODIPY, which is

a family of fluorophores based on the 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene moiety. For further information on fluorescent label moieties and fluorescence techniques, see, e.g., *Handbook of Fluorescent Probes and Research Chemicals*, by Richard P. Haughland, Sixth Edition, Molecular Probes, (1996), which is hereby incorporated by reference in its entirety. One of skill in the art can readily select a suitable labeling group, and conjugate such a labeling group to any of the compounds of the invention, without undue experimentation.

[0055] The term “quaternary nitrogen” refers to a tetravalent positively charged nitrogen atom including, for example, the positively charged nitrogen in a tetraalkylammonium group (e.g., tetramethylammonium, N-methylpyridinium), the positively charged nitrogen in protonated ammonium species (e.g., trimethyl-hydroammonium, N-hydropyridinium), the positively charged nitrogen in amine N-oxides (e.g., N-methyl-morpholine-N-oxide, pyridine-N-oxide), and the positively charged nitrogen in an N-amino-ammonium group (e.g., N-aminopyridinium).

[0056] Throughout the specification, unless otherwise noted, the nitrogen in the benzothiazepine ring of compounds of the present invention may optionally be a quaternary nitrogen. Non-limiting examples include ARM-113 and ARM-119.

[0057] The compounds described herein may exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

[0058] The term “prodrug” as employed herein denotes a compound that, upon administration to a subject, undergoes chemical conversion by metabolic or chemical processes to yield compounds of the present invention.

[0059] The term "compound(s) of the invention" as used herein means a compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, or any of the specific chemical compounds described herein, and salts, hydrates, complexes, metabolites, prodrugs and solvates thereof, or any combination thereof, such as may be used for the treatment or prevention of cardiac hypertrophy.

[0060] A "pharmaceutical composition" refers to a mixture of one or more of the compounds described herein, or pharmaceutically acceptable salts, hydrates or pro-drugs thereof, with other chemical components, such as physiologically acceptable carriers and excipients. The

purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0061] A "pro-drug" refers to an agent which is converted into the parent drug *in vivo*. Pro-drugs are often useful because, in some situations, they are easier to administer than the parent drug. They are bioavailable, for instance, by oral administration whereas the parent drug is not. The pro-drug also has improved solubility in pharmaceutical compositions over the parent drug. For example, the compound carries protective groups which are split off by hydrolysis in body fluids, *e.g.*, in the bloodstream, thus releasing active compound or is oxidized or reduced in body fluids to release the compound.

[0062] A compound of the present invention also can be formulated as a pharmaceutically acceptable salt, *e.g.*, acid addition salt, and complexes thereof. The preparation of such salts can facilitate the pharmacological use by altering the physical characteristics of the agent without preventing its physiological effect. Examples of useful alterations in physical properties include, but are not limited to, lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate administering higher concentrations of the drug.

[0063] The term "pharmaceutically acceptable salt" means a salt that is suitable for, or compatible with, the treatment of a patient or a subject such as a human patient. The salts can be any non-toxic organic or inorganic salt of any of the compounds represented by Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p or any of the specific compounds described herein, or any of their intermediates. Illustrative salt-forming ions include, but are not limited to, ammonium (NH_4^+), calcium (Ca^{2+}), iron (Fe^{2+} and Fe^{3+}), magnesium (Mg^{2+}), potassium (K^+), pyridinium ($C_5H_5NH^+$), quaternary ammonium (NR_4^+), sodium (Na^+), acetate, carbonate, chloride, bromide, citrate, cyanide, hydroxide, nitrate, nitrite, oxide, phosphate, sulfate, maleate, fumarate, lactate, tartrate, gluconate, besylate, and valproate. Illustrative inorganic acids that form suitable salts include, but are not limited to, hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable acid addition salts include, but are not limited to, mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as

well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either mono or di-acid salts can be formed, and such salts exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of an appropriate salt can be performed by one skilled in the art. For example, one can select salts in reference to “Handbook of Pharmaceutical Salts : Properties, Selection, and Use” by P. Heinrich Stahl and Camille G. Wermuth, or Berge (1977) “Pharmaceutcial Salts” J. Pharm Sci., Vol 66(1), p 1-19. Other non-pharmaceutically acceptable salts (*e.g.*, oxalates) may be used, for example, in the isolation of compounds of the invention for laboratory use or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

[0064] The compounds of the present invention form hydrates or solvates, which are included in the scope of the claims. When the compounds of the present invention exist as regioisomers, configurational isomers, conformers or diasteroisomeric forms all such forms and various mixtures thereof are included in the scope of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II. It is possible to isolate individual isomers using known separation and purification methods, if desired. For example, when a compound of the present invention is a racemate, the racemate can be separated into the (S)-compound and (R)-compound by optical resolution. Individual optical isomers and mixtures thereof are included in the scope of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II.

[0065] The term "solvate" as used herein means a compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, or a pharmaceutically acceptable salt thereof, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate."

[0066] The terms an "effective amount," "sufficient amount," “therapeutically effective amount,” or “prophylactically effective” amount” of an agent or compounds, as used herein, refer to amounts sufficient to effect the beneficial or desired results, including clinical results

and, as such, the actual "amount" intended will depend upon the context in which it is being applied, such as whether the desired clinical outcome is prevention or treatment. The term "effective amount" also includes that amount of the compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, which is "therapeutically effective" or "prophylactically effective" and which avoids or substantially attenuates undesirable side effects.

[0067] As used herein and as well understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Unless otherwise stated, the term "treatment" should be construed as encompassing preventive and therapeutic methods.

[0068] The terms "animal," "subject" and "patient" as used herein include all members of the animal kingdom including, but not limited to, mammals, animals (*e.g.*, cats, dogs, horses, etc.) and humans.

[0069] All stereoisomers of the compounds of the present invention (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this invention. Individual stereoisomers of the compounds of the invention may, for example, be substantially free of other isomers (e.g., as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present invention may have the S or R configuration as defined by the IUPAC 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

[0070] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 99% of the compound ("substantially pure" compound), which is then used or formulated as described herein. Such "substantially pure" compounds of the present invention are also contemplated herein as part of the present invention.

[0071] All configurational isomers of the compounds of the present invention are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present invention embraces both cis (*Z*) and trans (*E*) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings.

[0072] Throughout the specifications, groups and substituents thereof may be chosen to provide stable moieties and compounds.

<u>Prevention and Treatment of Cardiac Hypertrophy</u>

[0073] In one embodiment, the present invention provides compositions and methods that are useful for treating and/or preventing cardiac hypertrophy. More particularly, the present invention provides compositions comprising the compounds described herein, and methods of treatment and/or prevention comprising administration of these compositions to subjects suffering from, or at risk of developing cardiac hypertrophy.

[0074] In certain embodiments, the compositions and methods of the present invention may be used preventively in subjects who are not yet suffering from cardiac hypertrophy, but whom exhibit one or more "risk factors" for cardiac hypertrophy or are otherwise predisposed to the development of cardiac hypertrophy. Some factors that indicate a risk of developing or a predisposition to cardiac hypertrophy include, but are not limited to, chest pains, syncope, palpitations, effort dyspnea, aortic stenosis, coronary artery disease, heart failure, cardiomyopathy, myocarditis, hypertension, coarctation of the aorta, aortic regurgitation, mitral regurgitation, left-to-right shunts, restrictive cardiomyopathy, ischemic heart disease, pericardial tamponade, constrictive pericarditis, and restrictive cardiomyopathy. These same factors may be present in subjects who are suffering from cardiac hypertrophy.

[0075] Genetic factors may also be associated with a predisposition to development of cardiac hypertrophy or with the presence of cardiac hypertrophy in a subject. Cardiac hypertophy caused by a gene mutation is often referred to as "hypertrophic cardiomyopathy."

Examples of genes in which mutations may be associated with cardiac hypertophy include, but are not limited to, the beta cardiac myosin heavy chain gene, cardiac actin gene, cardiac troponin T gene, alpha-tropomyosin gene, cardiac troponin I gene, cardiac myosin binding protein C gene, the myosin light chain genes, genes encoding other sarcomere proteins, genes encoding proteins of ryanodine receptor complexes, and genes encoding proteins that affect the function of ryanodine receptor complexes.

[0076] The methods and compositions of the present invention may be used to treat or prevent any type of cardiac hypertrophy regardless of its cause. For example, the methods and compositions of the invention may be used to treat cardiac hypertrophy associated with hemodynamic overload, such as hemodynamic overload caused by systematic hypertension or an aortic stenosis. The methods and compositions of the invention may also be used to treat or prevent cardiac hypertrophy associated with myocardial infarction, congestive heart failure, idiopathic hypertrophic subaortic stenosis (IHSS), hypertrophic obstructive cardiomyopathy (HOCM), apical hypertrophic cardiomyopathy, non-obstructive hypertrophic cardiomyopathy, or some other type of chronic hemodynamic overload. In addition, the methods and compositions of the invention may also be used to treat or prevent cardiac hypertrophy associated with sudden cardiac death, or a cardiac arrhythmia, including, but not limited to, an arrhythmia selected from the group consisting of ventricular fibrillation, ventricular tachycardia, bradycardia, long QT syndrome, QT 455ms, and an exercise induced arrhythmia.

[0077] In preferred embodiments, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a subject having a mutation in a ryanodine receptor gene. In a more preferred embodiment, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a subject having a mutation in a RyR2 gene. More preferably still the subject has a mutation in a RyR2 gene that results in abnormal functioning of the ryanodine receptor, such as an increased open probability or "leakiness" of the ryanodine receptor or reduced binding to calstabin 2 (also known as FKBP12.6). The compositions and methods of the invention may be useful for reversing the "leakiness" caused by such mutations and thereby treating or preventing hypertrophy of the heart muscle.

[0078] In a particularly preferred embodiment, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a human subject having a mutation in the human RyR2 gene selected from the group consisting of the R929C mutation, the G2367 R mutation, the R2642K mutation and the E3654D mutation. These mutations are described below.

Mutations

[0079] In one embodiment, the present invention is directed to methods of treatment of subjects having one or more mutations in the human RyR2 gene that are associated with cardiac hypertrophy. Such mutations include any mutations in the RyR2 gene that alter the function of a RyR2 protein, such as mutations in the RyR2 protein that affect the interaction between a RyR2 protein and a calstabin protein and/or mutations that result in an increased open probability or the RyR channel. Such mutations may be point mutations or larger mutations. Such mutations may be missense mutations, such as conservative or non-conservative missense mutations.

[0080] In one embodiment, the present invention is directed to methods of treatment of subjects having a RyR2 R929C mutation, which is a mutation that results in an amino acid change in the RyR2 protein, namely a change from an arginine residue to a cysteine residue at amino acid position 929 of the RyR2 protein.

[0081] In another embodiment, the present invention is directed to methods of treatment of subjects having a RyR2 G2367R mutation, which is a mutation that results in an amino acid change in the RyR2 protein, namely a change from a glycine residue to an arginine residue at amino acid position 2367 of the RyR2 protein.

[0082] In another embodiment, the present invention is directed to methods of treatment of subjects having a RyR2 R2642K mutation, which is a mutation that results in an amino acid change in the RyR2 protein, namely a change from an arginine residue to a lysine residue at amino acod position 2642 of the RyR2 protein.

[0083] In one embodiment, the present invention is directed to methods of treatment of subjects having a RyR2 E3654D mutation, which is a mutation that results in an amino acid change in the RyR2 protein, namely a change from a glutamic acid residue to an aspartic acid residue at amino acod position 3654 of the RyR2 protein.

Subjects

[0084] In preferred embodiments, the compositions described herein are administered therapeutically or prophylactically to subjects who are suffering from, or at risk of developing cardiac hypertrophy. Such a subject may be any animal that is suffering from, or at risk of developing cardiac hypertrophy. For example, in one embodiment, the subject is a mammal. Examples of mammals that may be treated using the methods and compositions of the invention include, but ar enot limited to, primates, rodents, ovine species, bovine species, porcine species, equine species, feline species and canine species. In preferred embodiments the subjects are human.

[0085] In one aspect, the present invention encompasses compositions and methods for the treatment of subjects who have, or who are at risk of developing, overload cardiac hypertrophy, such as cardiac hypertrophy associated with systematic hypertension, aortic stenosis, myocardial infarction, congestive heart failure, idiopathic hypertrophic subaortic stenosis (IHSS), hypertrophic obstructive cardiomyopathy (HOCM), apical hypertrophic cardiomyopathy, non-obstructive hypertrophic cardiomyopathy, or some other type of chronic hemodynamic overload. The present invention also encompasses compositions and methods for the treatment of subjects who have, or who are at risk of developing, cardiac hypertrophy associated with sudden cardiac death, or a ventricular or atrial cardiac arrhythmia, such as a cardiac arrhythmia selected from the group consisting of ventricular fibrillation, ventricular tachycardia, bradycardia, long QT syndrome, QT 455ms, and exercise induced arrhythmias.

[0086] In another aspect the present invention encompasses compositions and methods for the treatment of subjects who have, or are at risk of developing, a form of cardiac hypertrophy caused by a mutation or a heritable genetic disorder, such as a hypertrophic cardiomyopathy. For example, the compositions and methods of the invention may be useful for the treatment of hypertrophic cardiomyopathy caused by mutations in genes including, but not limited to, beta cardiac myosin heavy chain genes, cardiac actin genes, cardiac troponin T genes, alpha-tropomyosin genes, cardiac troponin I genes, cardiac myosin binding protein C genes, myosin light chain genes, sarcomere protein genes, genes encoding proteins of ryanodine receptor complexes, and genes encoding proteins that affect the function of ryanodine receptor complexes.

[0087] In preferred embodiments, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a subject having a mutation in a ryanodine receptor gene. In a more preferred embodiment, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a subject having a mutation in a RyR2 gene. More preferably still the subject has a mutation in a RyR2 gene that results in defective functioning of the ryanodine receptor, such as an increased open probability or "leakiness" of the ryanodine receptor. Examples of such mutations include, but are not limited to, mutations in the RyR2 receptor that decrease the affinity of binding of calstabin2 (also known as FKBP12.6) to the ryanodine receptor. The compositions and methods of the invention may be useful for reversing the "leakiness" caused by such mutations and thereby treating or preventing hypertrophy of the heart muscle. In a particularly preferred embodiment, the methods and compositions of the invention may be used to treat or prevent cardiac hypertrophy in a human subject having a mutation in the human RyR2 gene selected from the group consisting of the R929C mutation, the G2367 R mutation, the R2642K mutation and the E3654D mutation.

[0088] In other embodiments, the "subjects" of the present invention may also be *in vitro* or *in vivo* systems, including, without limitation, isolated or cultured cells or tissues, *in vitro* assay systems.

Compositions

[0089] The compounds described herein may be formulated into compositions for administration to subjects for the treatment and/or prevention of cardiac hypertrophy. The compositions comprise one or more of the 1,4, benzothiazepine compounds described herein (such as the compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II,), in admixture with a pharmaceutically acceptable diluent and/or carrier and optionally one or more other pharmarceutically acceptable additives. The pharmaceutically-acceptable diluents and/or carriers and any other additives must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the subject to whom the composition will be administered. One of skill in the art can readily formulate the compounds of the invention into compositions suitable for administration to subjects, such as human subjects, for example using the teaching a standard text such as Remington's Pharmaceutical Sciences, 18th ed, (Mack Publishing Company: Easton, Pa., 1990), pp. 1635-36), and by taking into account the selected route of delivery.

[0090] Examples of diluents and/or carriers and/or other additives that may be included in the compostions of the invention include, but are not limited to, water, glycols, oils, alcohols, aqueous solvents, organic solvents, DMSO, saline solutions, physiological buffer solutions, peptide carriers, starches, sugars, preservatives, antioxidants, coloring agents, pH buffering agents, granulating agents, lubricants, binders, disintegrating agents, emulsifiers, binders, excipients, extenders, glidants, solubilizers, stabilizers, surface active agents, suspending agents, tonicity agents, viscosity-altering agents, carboxymethyl cellulose, crystalline cellulose, glycerin, gum arabic, lactose, magnesium stearate, methyl cellulose, powders, saline, sodium alginate. The combination of diluents and/or carriers and/or other additives used can be varied taking into account the nature of the active agents used (for example the solubility and stability of the active agents), the route of delivery (e.g. oral, parenteral, etc.), whether the agents are to be delivered over an extended period (such as from a controlled-release capsule), whether the agents are to be co-administered with other agents, and various other factors. One of skill in the art will readily be able to formulate the compounds for the desired use without undue experimentation.

Dosing & Administration

[0091] In accordance with a method of the present invention, the compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, may be administered to the subject (or contacted with cells of the subject) in an amount effective to treat or prevent cardiac hypertrophy, and/or in an amount effective to reduce calcium "leak" through the RyR channel, and/or in an amount effective to reduce the calcium current through the RyR channel, and/or in an amount effective to stabilize gating of the RyR channel, and/or in amount effective to increase the binding of calstabin to thr RyR complex in the subject, and/or in amount effective to reverse a malfunction of a RyR channel in the subject, particularly in the cardiac cells of the subject.

[0092] One of skill in the art can readily determine what would be an effective amount of the agents of the invention to be administered to a subject, taking into account whether the agent is being used prophylactically or therapeutically, and taking into account other factors such as the age, weight and sex of the subject, any other drugs that the subject may be taking, any allergies or contraindications that the subject may have, and the like. For example, an effective amount can be determined by the skilled artisan using known procedures, including analysis of titration curves established *in vitro* or *in vivo*. Also, where the desired subject is a

human, one of skill in the art can determine the effective dose from performing pilot experiments in suitable animal model species and scaling the doses up or down depending on the subjects weight etc. Effective amounts can also be determined by performing clinical trials in individuals of the same species as the subject, for example starting at a low dose and gradually increasing the dose and monitoring the effects on cardiac hypertophy. Appropriate dosing regimens can also be determined by one of skill in the art without undue experimentation, in order to determine, for example, whether to administer the agent in one single dose or in multiple doses, and in the case of multiple doses, to determine an effective interval between doses.

[0093] In one embodiment, an effective amount of the compounds of the invention to administer to a subject ranges from about 0.01 mg/kg/day to about 20 mg/kg/day, and/or is an amount sufficient to achieve plasma levels ranging from about 300 ng/ml to about 1000 ng/ml. In one embodiment, the amount of compounds from the invention ranges from about 5 mg/kg/day to about 20 mg/kg/day. In another embodiment, from about 10 mg/kg/day to about 20 mg/kg/day is administered. In another embodiment, from about 0.01 mg/kg/day to about 10 mg/kg/day is administered. In another embodiment, from about 0.01 mg/kg/day to about 5 mg/kg/day is administered. In another embodiment, from about 0.05 mg/kg/day to about 5 mg/kg/day is administered. In another, preferred embodiment, from about 0.05 mg/kg/day to about 1 mg/kg/day is administered.

[0094] The compositions described herein may be administered to a subject by any suitable method that allows the agent to exert its effect on the subject *in vivo*. For example, the compositions may be administered to the subject by known procedures including, but not limitated to, by oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation, via nasal delivery, vaginally, rectally, and intramuscularly. The compounds of the invention may be administered parenterally, or by epifascial, intracapsular, intracutaneous, subcutaneous, intradermal, intrathecal, intramuscular, intraperitoneal, intrasternal, intravascular, intravenous, parenchymatous, or sublingual delivery. Delivery may be by injection, infusion, catheter delivery, or some other means, such as by tablet or spray. In one embodiment, the agent is adiminstered to the subject by way of delivery directly to the heart tissue, such as by way of a catheter inserted into, or in the proximity of the subject's heart, or by using delivery vehicles capable of targeting the drug to the heart. For example, the compounds of the invention may

be conjugated to or administered in conjunction with an agent that is targeted to the heart, such as an antibody or antibody fragment.

[0095] For oral administration, a formulation of the compounds of the invention may be presented as capsules, tablets, powders, granules, or as a suspension or solution. The formulation may contain conventional additives, such as lactose, mannitol, cornstarch or potato starch, binders, crystalline cellulose, cellulose derivatives, acacia, cornstarch, gelatins, disintegrators, potato starch, sodium carboxymethylcellulose, dibasic calcium phosphate, anhydrous or sodium starch glycolate, lubricants, and/or or magnesium stearate.

[0096] For parenteral administration (*i.e.*, administration by through a route other than the alimentary canal), the compounds of the invention may be combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation may be prepared by dissolving the active ingredient in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering the solution sterile. The formulation may be presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation may be delivered by injection, infusion, or other means known in the art.

[0097] For transdermal administration, the compounds of the invention may be combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, *N*-methylpyrrolidone and the like, which increase the permeability of the skin to the compounds of the invention and permit the compounds to penetrate through the skin and into the bloodstream. The compositions also may be further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinylacetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which are dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

[0098] In some embodiments, the composition is in unit dose form such as a tablet, capsule or single-dose injection or infusion vial.

[0099] In certain embodiments, the agents described herein may be used in combination with other agents useful for the treatment of cardiac hypertrophy or with other agents that

ameliorate the effect of certain risk factors for cardiac hypertrophy. For example, in one embodiment, the agents of the invention may be delivered to a subject as part of a composition containing one or more additional active agents. In another embdodiment, the agents of the invention may be delivered to a subject in a composition or formulation containing only that active agent, while one or more other agents useful for the treatment of cardiac hypertrophy may be also be administered to the subject in one or more separate compositions or formulations.

[00100] The agents of the invention and the other agents useful for the treatment of cardiac hypertrophy may be administered to the subject at the same time, or at different times. For example, the agents of the invention and the other agents may be administered within minutes, hours, days, weeks, or months of each other, for example as part of the overall treatment regimen of a subject.

[00101] Examples of the types of anti-hypertrophic agents that may be used in combination with the agents of the invention include, but are not limited to, β-adrenergic blockers, calcium channel blockers and anti-arrhythmic drugs.

[00102] The agents of the invention may also be used in combination with surgical or other interventional treatment regimens used for the treatment of cardiac hypertrophy, including, but not limited to, septal myotomy, myormectomy and mitral valve replacement surgery.

Screening for new compounds useful for treating cardiac hypertrophy

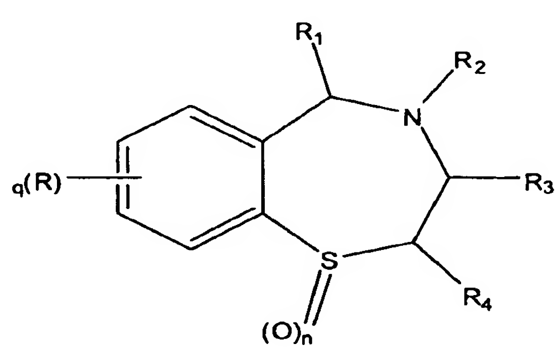
[00103] In another embodiment, the present invention is directed to methods for identifying additional compounds that may be useful for the treatment of cardiac hypertrophy. Such methods may be based on, *inter alia*, identifying compounds that increase binding of calstabins to RyRs, and/or decrease the calcium current through RyR channels, and the like. Examples of suitable assays and screening methods that may be used to identify new compounds that may be useful for the treatment of cardiac hypertrophy are described in U.S. patent applications 09/568,474, 10/288,606, 10/680,988, 10/608,723, 10/809,089, 10/763,498, 10/794,218, 11/088,058, 11/088,123, 11/212,309, 11/506,285, and 11/212,413, the contents of which are hereby incorporated by reference.

Compounds

[00104] The present invention encompasses compounds useful for the treatment and/or prevention of cardiac hypertrophy, and methods of treatment and/or prevention comprising administration of such compounds, or compositions containing such compounds, to subjects who are suffereing from, or who are at risk of developing, cardiac hypertrophy. The compounds of the invention decrease the open probability of RyR receptor channels, particularly PKA phosphorylated RyR channels, and thereby decrease the Ca^{2+} current through such channels. The compounds of the invention exert this effect, at least in part, by increasing the affinity with which calstabin proteins bind to RyRs, and/or by inhibiting a decrease in binding of calstabins to RyRs, and/or by inhibiting dissociation of calstabins from RyRs, particularly PKA phosphorylated RyRs. The compounds of the invention decrease the open probability of RyR channels and decrease the "leak" of Ca^{2+} through such channels.

[00105] The present invention relates to use of 1,4, benzothiazepine compounds in the treatment of cardiac hypertrophy. In one embodiment, the present invention provides N-substituted 1,4, benzothiazepines, such as the N-substituted 1,4, benzothiazepine compound known as JTV-519 or K-201. In preferred embodiments, the present invention provides 1,4, benzothiazepine compounds as described by the chemical formulae Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II,, as described below.

[00106] In one aspect, the present invention provides methods for the treatment or prevention of cardiac hypertrophy which comprise administering compounds of Formula I to subjects in need thereof. In another aspect, the present invention provides compositions useful for the treatment or prevention of cardiac hypertrophy which comprise compounds of Formula I. The structure of Formula I is as follows:



and wherein m is 0, 1, 2, 3, or 4

wherein,

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, -O-acyl, alkyl, alkoxyl, alkylamino, alkylarylamino, alkylthio, cycloalkyl, alkylaryl, aryl, heteroaryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be optionally substituted;

R_1 is selected from the group consisting of H, oxo, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_2 is selected from the group consisting of H, $-C(=O)R_5$, $-C(=S)R_6$, $-SO_2R_7$, $-P(=O)R_8R_9$, $-(CH_2)_m-R_{10}$, alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, alkylaryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be optionally substituted and wherein m is 0, 1, 2, 3, or 4;

R_3 is selected from the group consisting of H , $-CO_2Y$, -C(=O)NHY, acyl, -O-acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted; and wherein Y is selected from the group consisting of H, alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl, and wherein each alkyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_4 is selected from the group consisting of H, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl; wherein each alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, heteroaryl, and heterocyclyl may be optionally substituted;

R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-C(=O)NHNR_{15}R_{16}$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-CH_2X$, acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted, and wherein z is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-OR_{15}$, $-NHNR_{15}R_{16}$, -NHOH, $-NR_{15}R_{16}$, $-CH_2X$, acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_7 is selected from the group consisting of $-OR_{15}$, $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-CH_2X$, alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkenyl, alkynyl, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_8 and R_9 independently are selected from the group consisting of OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, $C(=O)(R_{12})$, $NHC=O(R_{12})$, $-OC=O(R_{12})$, and $-P(=O)R_{13}R_{14}$;

R_{11}, R_{12}, R_{13}, and R_{14} independently are selected from the group consisting of H, OH, NH_2, $-NHNH_2$, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted;

X is selected from the group consisting of halogen, -CN, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, alkylaryl, cycloalkyl, cycloalkylalkyl, heteroaryl, heterocyclyl, and heterocyclylalkyl may be optionally substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted;

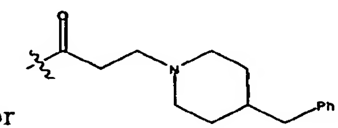
the nitrogen in the benzothiazepine ring may optionally be a quaternary nitrogen; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof.

[00107] In certain embodiments, the present invention uses compounds of Formula I, with the proviso that when q is 0 and n is 0, then R_2 is not H, Et, -C(=O)NH_2, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH_2Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH_2, -S(=O)$_2$Me, or -S(=O)$_2$Et;

further provided that when q is 0 and n is 1 or 2, then R_2 is not -C(=O)Me, -C(=O)Et, -S(=O)$_2$Me, or -S(=O)$_2$Et;

further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, -S(=O)$_2$Me, or -S(=O)$_2$Et; and

further provided that when q is 1, n is 0, and R is OCT_3, OH, C_1-C_3 alkoxyl at the 7 position of the benzothiazepene ring, then R_2 is not H, -C(=O)CH=CH_2, or .

[00108] Examples of compounds that may be used in conjunction with the invention include, without limitation, S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, as herein defined. In certain embodiments, the compounds are isolated and substantially pure.

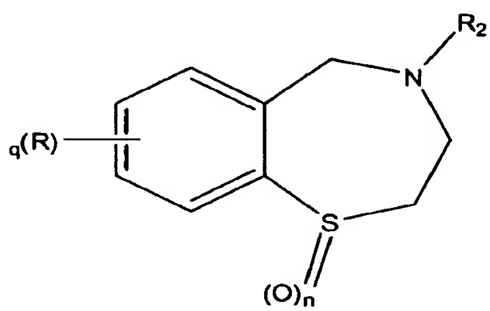
[00109] In another embodiment, the present invention uses compounds of Formula I, as described above, with the proviso that the compound is not S4, S7, S20, S24, S25, S26, S27, or S36.

[00110] In another embodiment, the present invention uses compounds of Formula I, as described above, with the proviso that the compound is not S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44,

S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, or S100.

[00111] In another embodiment, the present invention uses 1,4, benzothiazepine compounds, such as compounds of Formula I, with the proviso that the compound is not JTV-519.

[00112] In one embodiment, the present invention provides methods and uses which comprise administering compounds of Formula **I-a:**



(I-a)

wherein:

n is 0, 1, or 2;

q is 0, 1, 2, 3, or 4;

each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted or unsubstituted;

R_2 is selected from the group consisting of H, $-C=O(R_5)$, $-C=S(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, $-(CH_2)_m-R_{10}$, alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl; wherein each alkyl, aryl, heteroaryl, cycloalkyl, cycloalkylalkyl, and heterocyclyl may be substituted or unsubstituted, wherein m is 0, 1, 2, 3, or 4;

R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-C(=O)NHNR_{15}R_{16}$, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-CH_2X$, acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted, and wherein z is 1, 2, 3, 4, 5, or 6;

R_6 is selected from the group consisting of $-OR_{15}$, $-NHNR_{15}R_{16}$, -NHOH, $-NR_{15}R_{16}$, $-CH_2X$, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_7 is selected from the group consisting of H, $-OR_{15}$, $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, -CH_2X, alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, alkynyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_8 and R_9 independently are selected from the group consisting of -OH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

R_{10} is selected from the group consisting of $-NR_{15}R_{16}$, OH, $-SO_2R_{11}$, $-NHSO_2R_{11}$, -$C(=O)R_{12}$, $-NH(C=O)R_{12}$, $-O(C=O)R_{12}$, and $-P(=O)R_{13}R_{14}$;

R_{11}, R_{12}, R_{13}, and R_{14} independently are selected from the group consisting of H, OH, NH_2, -$NHNH_2$, -NHOH, acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

X is selected from the group consisting of halogen, -CN, $-CO_2R_{15}$, $-C(=O)NR_{15}R_{16}$, $-NR_{15}R_{16}$, $-OR_{15}$, $-SO_2R_7$, and $-P(=O)R_8R_9$; and

R_{15} and R_{16} independently are selected from the group consisting of H, acyl, alkenyl, alkoxyl, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkoxyl, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted; and

optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted or unsubstituted;

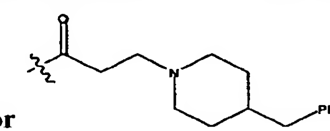
the nitrogen in the benzothiazepine ring may be optionally a quaternary nitrogen; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, and prodrugs thereof.

[00113] In one embodiment, it is provided that when q is 0 and n is 0, then R_2 is not H, Et, -C(=O)NH_2, (=O)NHPh, -C(=S)NH-nButyl, -C(=O)NHC(=O)CH_2Cl, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)CH=CH_2, -S(=O)$_2$Me, or -S(=O)$_2$Et;

further provided that when q is 0 and n is 1 or 2, then R_2 is not -C(=O)Me, -C(=O)Et, -S(=O)$_2$Me, or -S(=O)$_2$Et;

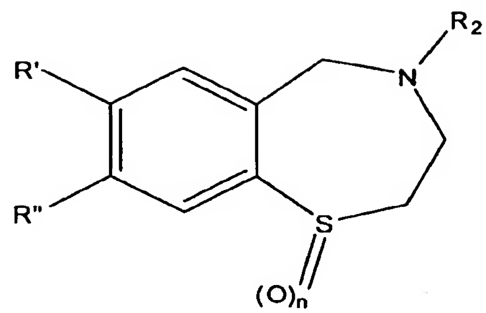
further provided that when q is 1, and R is Me, Cl, or F at the 6 position of the benzothiazepene ring, then R_2 is not H, Me, -C(=O)H, -C(=O)Me, -C(=O)Et, -C(=O)Ph, -S(=O)$_2$Me, or -S(=O)$_2$Et; and

further provided that when q is 1, n is 0, and R is OCT_3, OH, C_1-C_3 alkoxyl at the 7 position of the benzothiazepene ring, then R_2 is not H, -C(=O)CH=CH_2, or .

[00114] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-a**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, -NH_2, -NO_2, -CN, -CF_3, -OCF_3, -N_3, -S(=O)$_2$$C_1$-$C_4$alkyl, -S(=O)$C_1$-$C_4$alkyl, -S-$C_1$-$C_4$alkyl, -OS(=O)$_2$$CF_3$, Ph, -NH$CH_2$Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00115] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-a**, wherein R_2 is selected from the group consisting of –C=O(R_5), –C=S(R_6), -SO_2R_7, –P(=O)R_8R_9, and -$(CH_2)_m$-R_{10}, wherein m is 0, 1, 2, 3, or 4.

[00116] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-b**:



(I-b)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

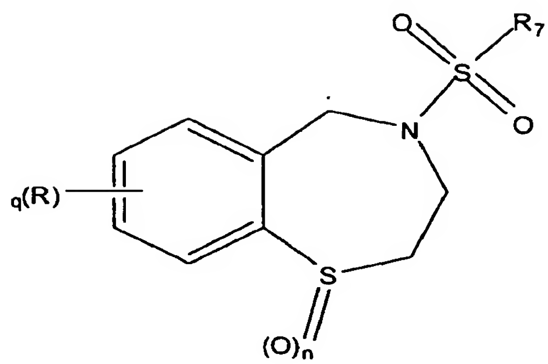
R_2 and n are as defined in compounds of formula **I-a** above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00117] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-b**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00118] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-b**, wherein R_2 is selected from the group consisting of $-C{=}O(R_5)$, $-C{=}S(R_6)$, $-SO_2R_7$, $-P(=O)R_8R_9$, and $-(CH_2)_m$-R_{10}.

[00119] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds formula of **I-c**:



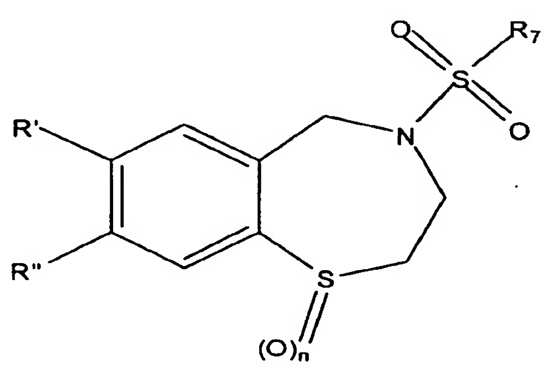
(I-c)

wherein each R, R_7, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites, and pro-drugs thereof.

[00120] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-c**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, -S-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00121] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-c**, wherein R_7 is selected from the group consisting of -OH, $-NR_{15}R_{16}$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00122] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-d:**



(I-d)

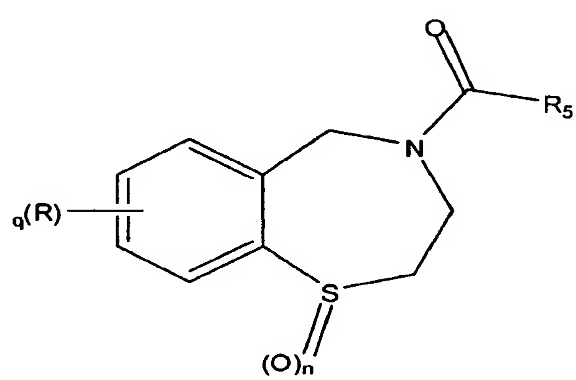
wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_7 and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00123] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-d**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00124] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-d**, wherein R_7 is selected from the group consisting of -OH, $-NR_{15}R_{16}$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, akenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00125] In one embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-e**:



(I-e)

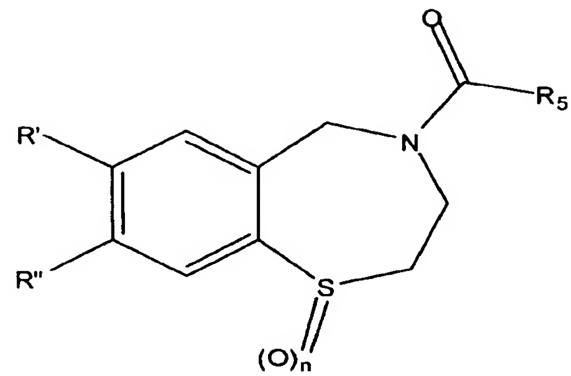
wherein each R, R_5, q and n is as defined compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00126] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, -S-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00127] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein R_5 is selected from the group consisting of $-NR_{15}R_{16}$, $-(CH_2)_zNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00128] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-e**, wherein R_5 is an alkyl substituted by at least one labeling group, such as a fluorescent, a bioluminescent, a chemiluminescent, a colorimetric and a radioactive labeling group. A fluorescent labeling group can be selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00129] In another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-f**:



(I-f)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

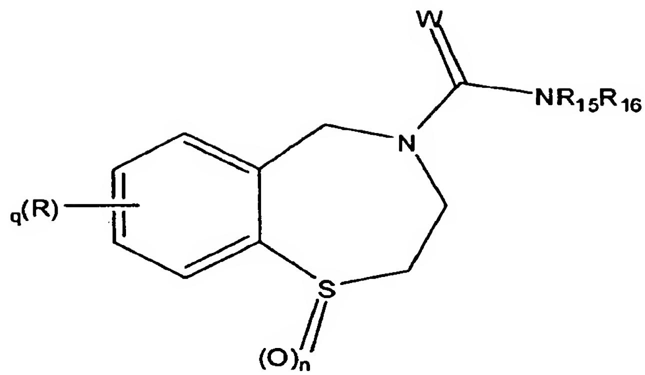
R_5 and n are as defined in compounds of formula **I-a** above;

and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00130] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-f**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S-C_1$-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00131] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-f**, wherein $-(CH_2)_zNR_{15}R_{16}$, selected from the group consisting of $-NR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkyl, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted.

[00132] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-g**:



(I-g)

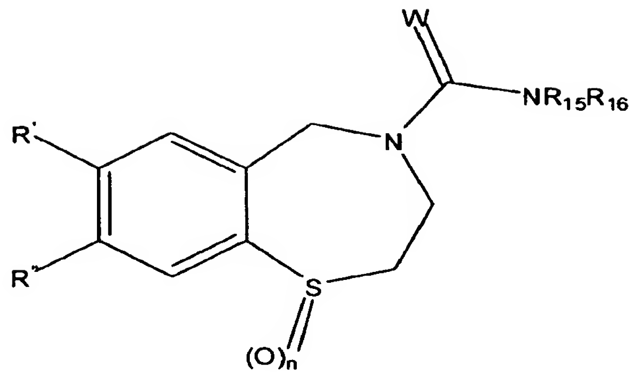
wherein W is S or O; each R, R_{15}, R_{16}, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00133] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, -S-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00134] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein R_{15} and R_{16} independently are selected from the group consisting of H, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00135] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein W is O or S.

[00136] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-h**:



(I-h)

wherein W is S or O;

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{15}, R_{16} and n are as defined in compounds of formula **I-a** above;

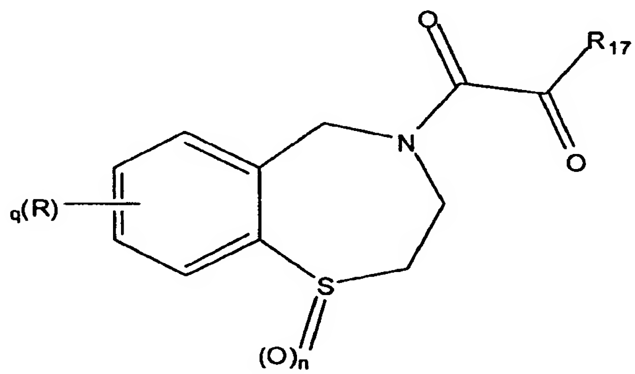
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00137] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-h**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S-C_1$-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00138] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-h**, wherein R_{15} and R_{16} independently are selected from the group consisting of H, OH, NH_2, alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkyl, alkylamino, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted; and optionally R_{15} and R_{16} together with the N to which they are bonded may form a heterocycle which may be substituted.

[00139] In some embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-g**, wherein W is O or S.

[00140] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-i**:



(I-i)

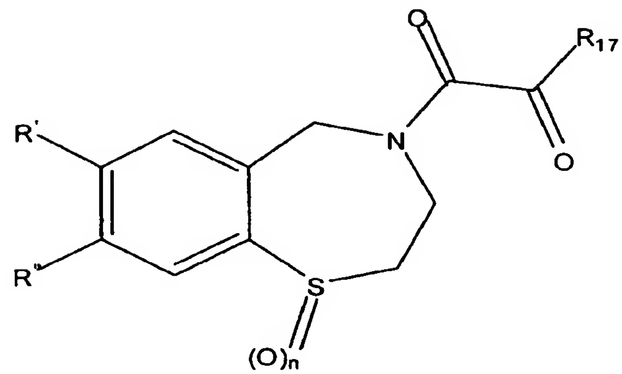
wherein R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

each R, q, and n is as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00141] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-i**, wherein each R is independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S-C_1$-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1, or 2.

[00142] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-i**, wherein R_{17} is $-NR_{15}R_{16}$, and $-OR_{15}$. In certain other embodiments, R_{17} is -OH, -OMe, -NEt, -NHEt, -NHPh, $-NH_2$, or $-NHCH_2$pyridyl.

[00143] In one embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-j**:



(I-j)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{17} is selected from the group consisting of $-NR_{15}R_{16}$, $-NHNR_{15}R_{16}$, -NHOH, $-OR_{15}$, $-CH_2X$, alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each alkenyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted;

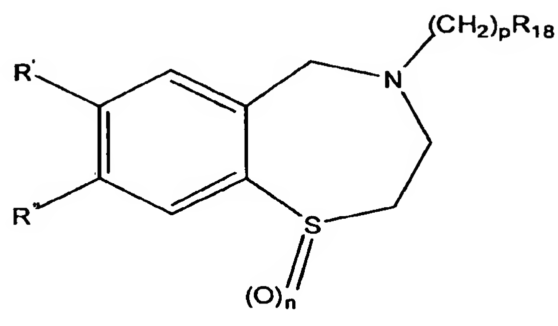
n is as defined in compounds of formula **I-a**; and

enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00144] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-j**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S$-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00145] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-j**, wherein R_{17} is $-NR_{15}R_{16}$ or $-OR_{15}$. In certain other embodiments, R_{17} is -OH, -OMe, -NEt, -NHEt, -NHPh, $-NH_2$, or -$NHCH_2$pyridyl.

[00146] In another embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-k**:



(I-k)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_{18} is selected from the group consisting of $-NR_{15}R_{16}$, $-C(=O)NR_{15}R_{16}$, $-(C=O)OR_{15}$, $-OR_{15}$, alkyl, aryl, cycloalkyl, heterocyclyl, and at one labeling group; wherein each alkyl, aryl, cycloalkyl, and heterocyclyl may be substituted or unsubstituted;

wherein p is 1, 2, 3, 4, 5, 6, 7, 8 9, or 10;

and n is 0, 1, or 2;

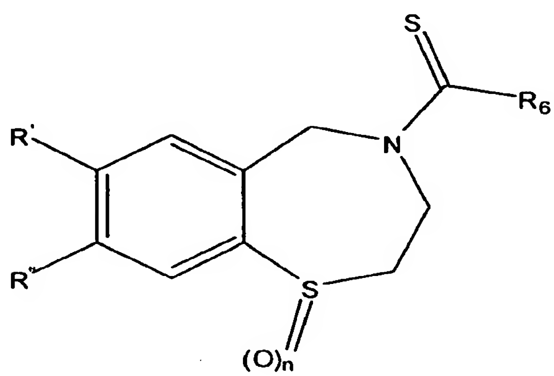
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00147] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-k**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S-C_1$-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00148] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-k**, wherein R_{18} is selected from the group consisting of $-NR_{15}R_{16}$, $-(C=O)OR_{15}$, $-OR_{15}$, alkyl, aryl, and at one labeling group; and

wherein each alkyl and aryl may be substituted or unsubstituted. In some cases, m is 1, and R_{18} is Ph, C(=O)OMe, C(=O)OH, aminoalkyl, NH_2, NHOH, or NHCbz. In other cases, m is 0, and R_{18} is C_1-C_4 alkyl, such as Me, Et, propyl, and butyl. In yet other cases, m is 2, and R_{18} is pyrrolidine, piperidine, piperazine, or morpholine. In some embodiments, m is 3, 4, 5, 5, 7, or 8, and R_{18} is a fluorescent labeling group selected from bodipy, dansyl, fluorescein, rhodamine, Texas red, cyanine dyes, pyrene, coumarins, Cascade Blue™, Pacific Blue, Marina Blue, Oregon Green, 4',6-Diamidino-2-phenylindole (DAPI), indopyra dyes, lucifer yellow, propidium iodide, porphyrins, arginine, and variants and derivatives thereof.

[00149] In yet another embodiment, the present invention provides methods and uses which comprise administering compounds of formula of **I-l**:



(I-l)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-SO_3H$, $-S(=O)_2$alkyl, -S(=O)alkyl, $-OS(=O)_2CF_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

R_6 and n are as defined in compounds of formula **I-a**;

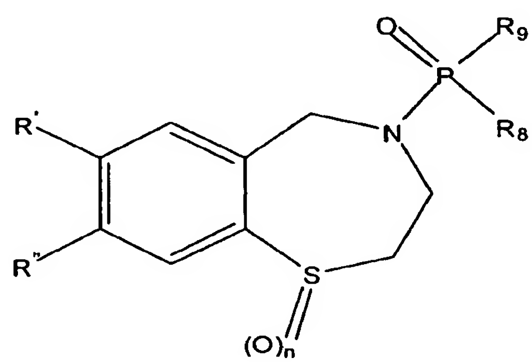
and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00150] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-l**, wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, $-S-C_1$-C_4alkyl, $-OS(=O)_2CF_3$,

Ph, -NHCH$_2$Ph, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00151] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-l**, wherein R_6 is selected from the group consisting of -$NR_{15}R_{16}$, -$NHNR_{15}R_{16}$, -OR_{15}, -NHOH, -CH$_2$X, acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, alkenyl, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted or unsubstituted. In some cases, R_6 is -$NR_{15}R_{16}$ such as –NHPh, pyrrolidine, piperidine, piperazine, morpholine, and the like. In some other cases, R_6 is alkoxyl, such as –O-tBu.

[00152] In a further embodiment, the present invention provides methods and uses which comprise administering compounds of formula **I-m**:



(I-m)

wherein R' and R" are independently selected from the group consisting of H, halogen, -OH, -NH$_2$, -NO$_2$, -CN, -CF$_3$, -OCF$_3$, -N$_3$, -SO$_3$H, -S(=O)$_2$alkyl, -S(=O)alkyl, -OS(=O)$_2$CF$_3$, acyl, alkyl, alkoxyl, alkylamino, alkylthio, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; and wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, aryl, heterocyclyl, heterocyclylalkyl, alkenyl, alkynyl, (hetero-)aryl, (hetero-)arylthio may be substituted or unsubstituted;

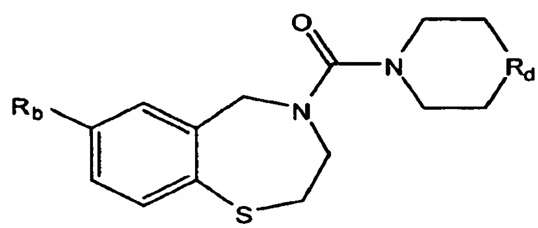
R_8, R_9 and n are as defined in compounds of formula **I-a** above; and enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes and pro-drugs thereof.

[00153] In certain embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-m**, wherein R' and R" are

independently selected from the group consisting of H, halogen, -OH, OMe, $-NH_2$, $-NO_2$, -CN, $-CF_3$, $-OCF_3$, $-N_3$, $-S(=O)_2C_1$-C_4alkyl, $-S(=O)C_1$-C_4alkyl, -S-C_1-C_4alkyl, $-OS(=O)_2CF_3$, Ph, $-NHCH_2Ph$, -C(=O)Me, -OC(=O)Me, morpholinyl and propenyl; and n is 0, 1 or 3. In some cases, R' is H or OMe, and R" is H.

[00154] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-m**, wherein R_8 and R_9 are independently alkyl, aryl, -OH, alkoxyl, or alkylamino. In some cases, R_8 is C_1-C_4 alkyl such as Me, Et, propyl and butyl; and R_9 is aryl such as phenyl.

[00155] In other embodiments, the present invention provides methods and uses which comprise administering compounds of formula **I-n**,



I-n

wherein:

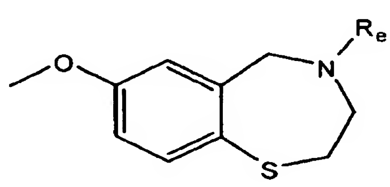
R_d is CH_2, or NR_a; and

R_a is H, –(C_1-C_6 alkyl)-aryl, wherein the aryl is a disubstituted phenyl or a benzo[1,3]dioxo-5-yl group, or an amine protecting group (e.g., a Boc group); and

R_b is hydrogen of alkoxy (e.g., methoxy).

[00156] Representative compounds of Formula **I-n** include without limitation S101, S102, S103, S114.

[00157] In certain other embodiments, the invention provides compounds of Formula **I-o**:



I-o

wherein:

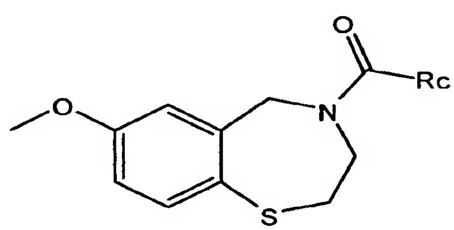
R_c is -(C_1-C_6 alkyl)-phenyl, -(C_1-C_6 alkyl)-C(O)R_b, or substituted or unsubstituted -C_1-C_6 alkyl; and

R_b is –OH or –O-(C_1-C_6 alkyl), and

wherein the phenyl or substituted alkyl is substituted with one or more of halogen, hydroxyl, -C_1-C_6 alkyl, -O-(C_1-C_6 alkyl), -NH_2, -NH(C_1-C_6 alkyl), -N(C_1-C_6 alkyl)$_2$, cyano, or dioxolane.

[00158] Representative compounds of Formula **I-o** include without limitation S107, S110, S111, S120, and S121.

[00159] In certain other embodiments, the invention provides compounds of Formula **I-p**:



I-p

wherein:

R_c is –(C_1-C_6 alkyl)-NH_2, -(C_1-C_6 alkyl)-OR_f, wherein R_f is H or –C(O)-(C_1-C_6)alkyl, or –(C_1-C_6 alkyl)-NHR_g wherein Rg is carboxybenzyl. Representative compound of Formula **I-p** include without limitation S109, S122, S123.

[00160] In non-limiting examples, Formulae-Ia, Ib, Ie, If, Ig, Ih, In are represented by compounds S101, S102, S103. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ii, Ij are represented by compound S104. In a non-limiting example, Formulae Ia, Ib, Io are represented by S107. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S108. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S109. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S110. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S111. In a non-limiting example, Formulae Ia, Ib,

Ic, Id are represented by S112. In a non-limiting example, Formulae Ia, Ib are represented by S113. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ig, Ih are represented by S114. In a non-limiting example, Formulae Ia, Ib, Ig, Ih, Il are represented by S115. In a non-limiting example, Formulae Ia, Ib, Ig, Ih, are represented by S116. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S117. In a non-limiting example, Formulae Ia, Ib, Ie, If are represented by S118. In a non-limiting example, Ia, Ib are represented by S119. In a non-limiting example, Formulae Ia, Ib, Ik, Io are represented by S120. In a non-limiting example, Formulae Ia, Ib, Ik, Io, Ip are represented by S121. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S122. In a non-limiting example, Formulae Ia, Ib, Ie, If, Ip are represented by S123.

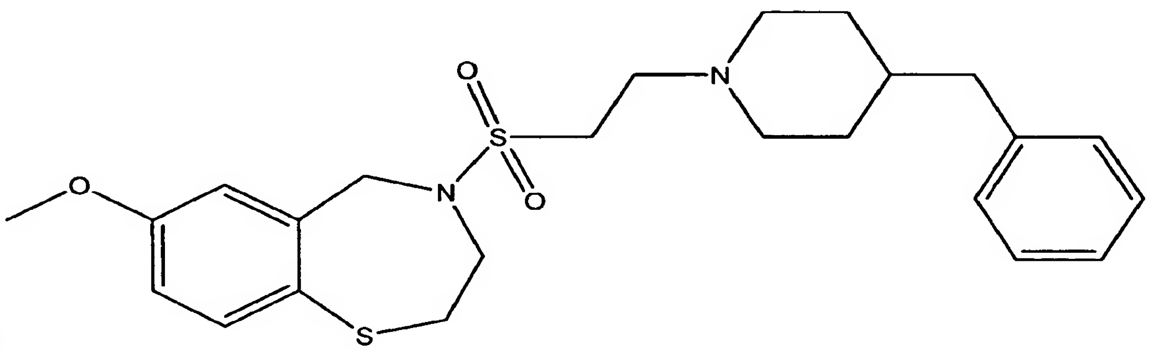
[00161] The compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, and Formula II can be used in methods that treat or prevent cardiac hypertrophy, and may also be used in compositions suitable for the treatment or prevention of cardiac hypertrophy. In one preferred embodiment, the compounds used have structures as described by Formula I-a, I-b, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-n, I-o, or I-p.

[00162] Examples of compounds that may be used in conjunction with the invention include, without limitation, S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123, as herein defined. In certain embodiments, the compounds are isolated and substantially pure.

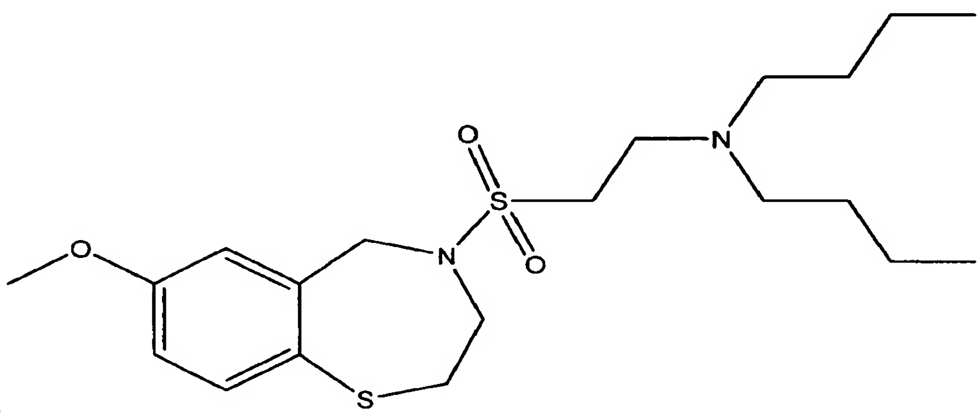
[00163] . In a certain embodiment of the methods the compound is not S4. In another embodiment, the compound is not S7. In another embodiment, the compound is not S8. In another embodiment, the compound is not S10. In another embodiment, the compound is not S20. In another embodiment, the compound is not S24. In another embodiment, the compound is not S25. In another embodiment, the compound is not S26. In another embodiment, the compound is not S27. In another embodiment, the compound is not S36. In

another embodiment, the compound is not any one of S1-100. In another embodiment, the compound is not JTV-519.

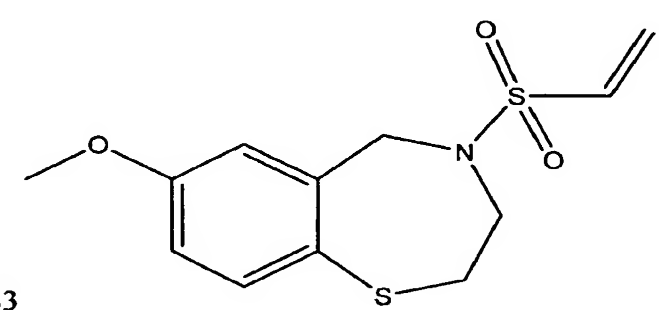
[00164] The named "S" compounds described herein have the following structures:



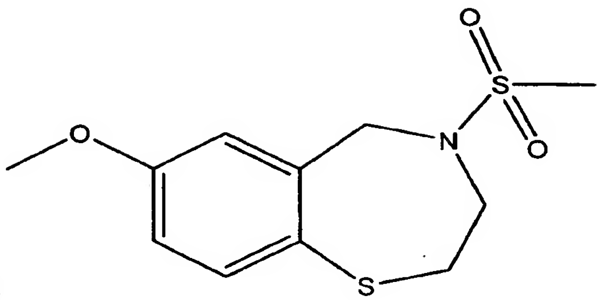
S1



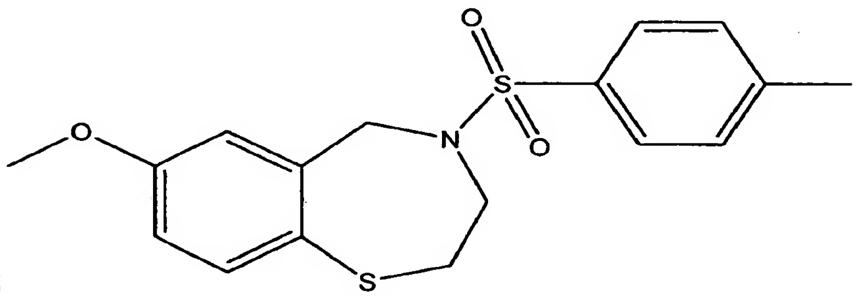
S2



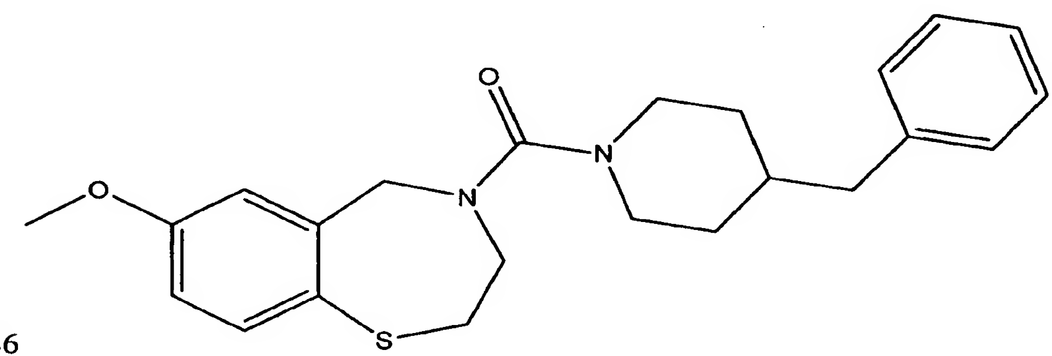
S3



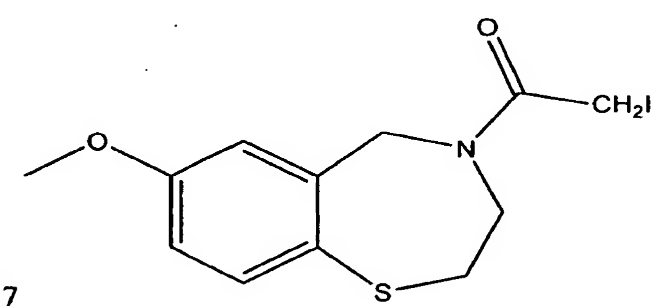
S4



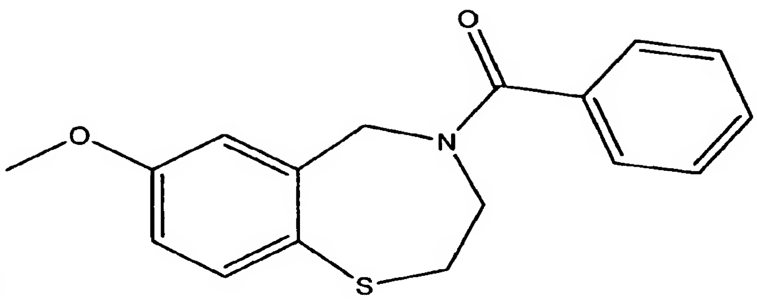
S5



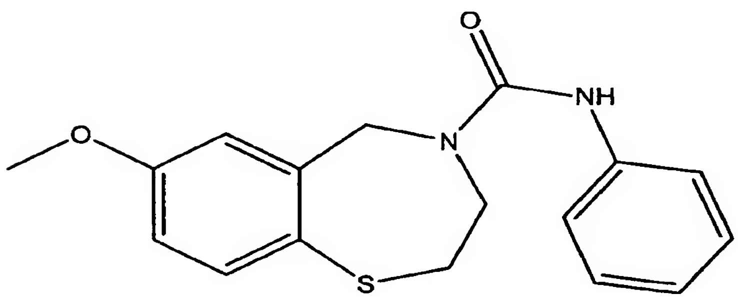
S6



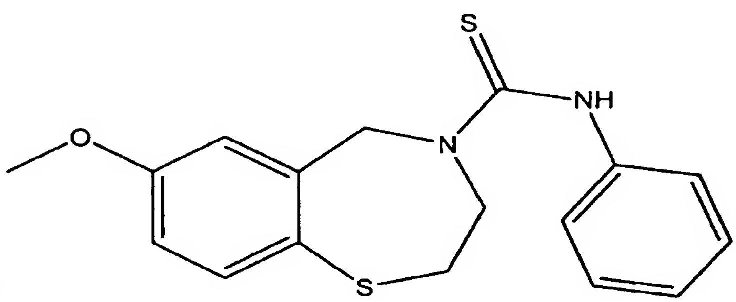
S7



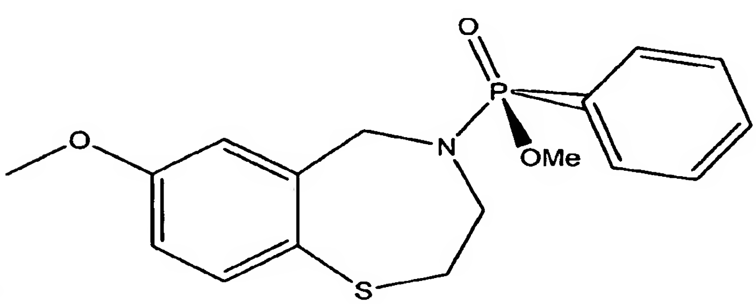
S9



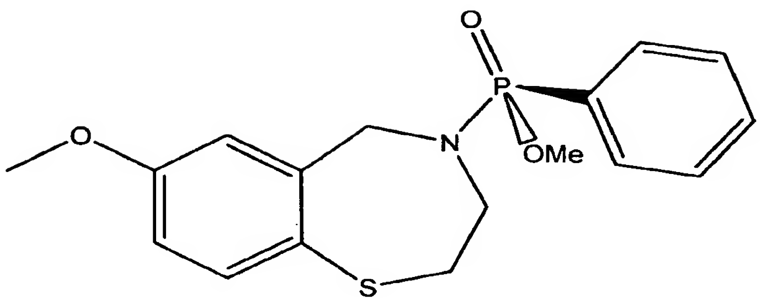
S11



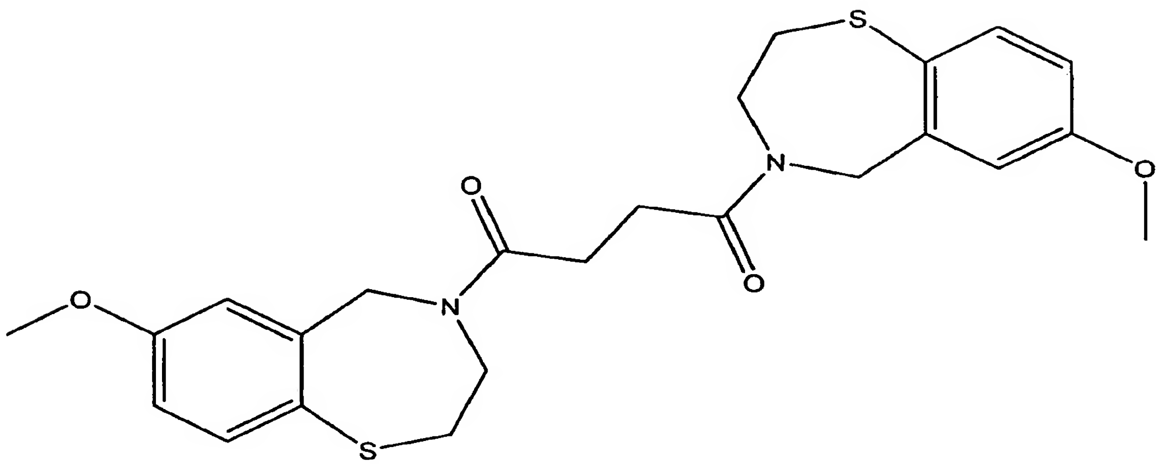
S12



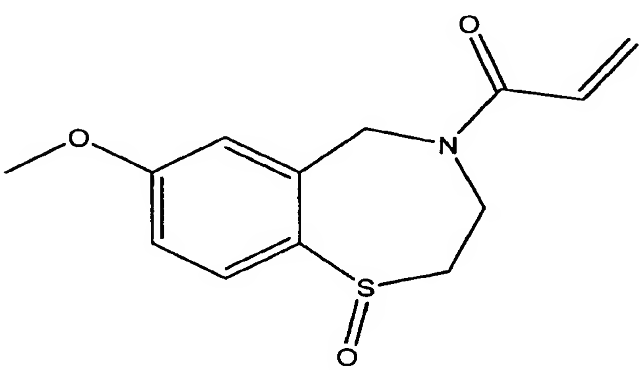
S13



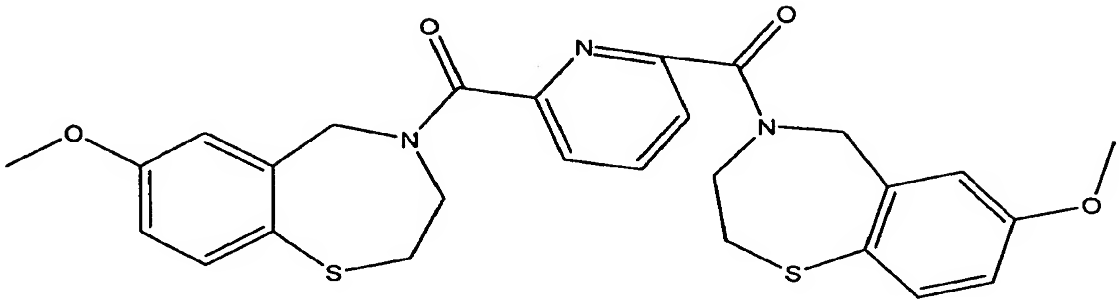
S14



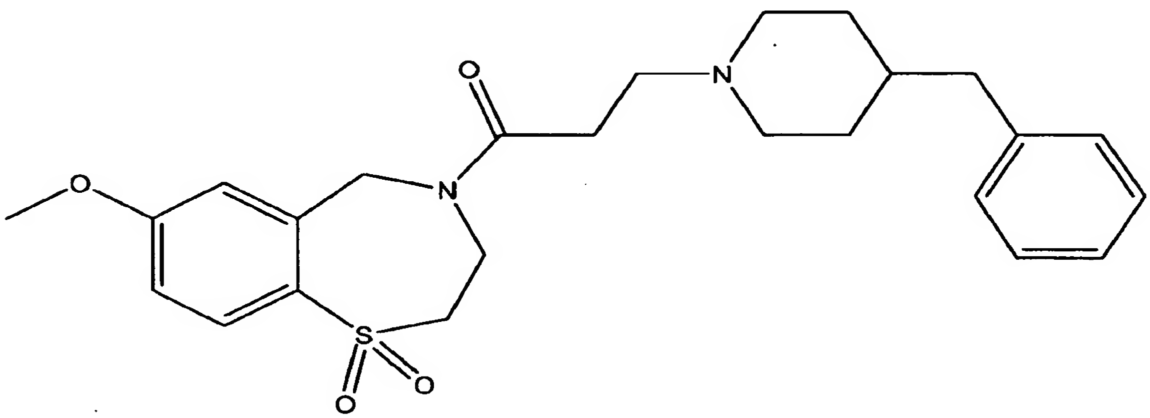
S19



S20

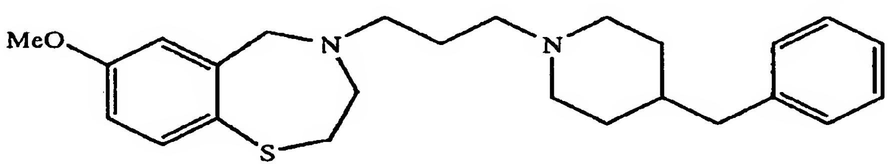


S22

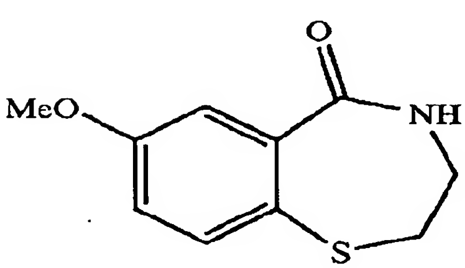


S23

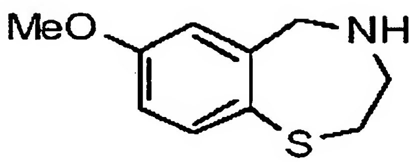
S-24



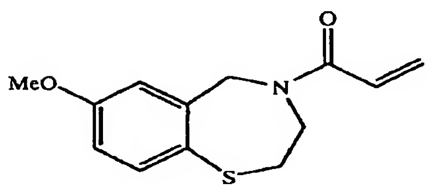
S24



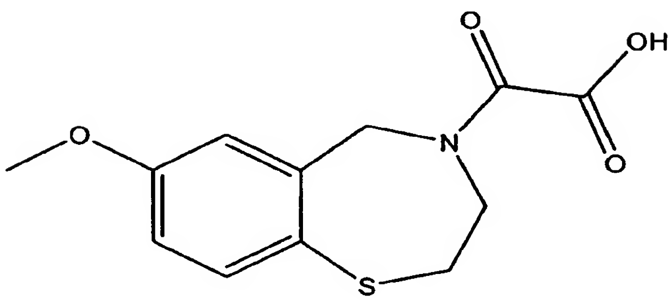
S25



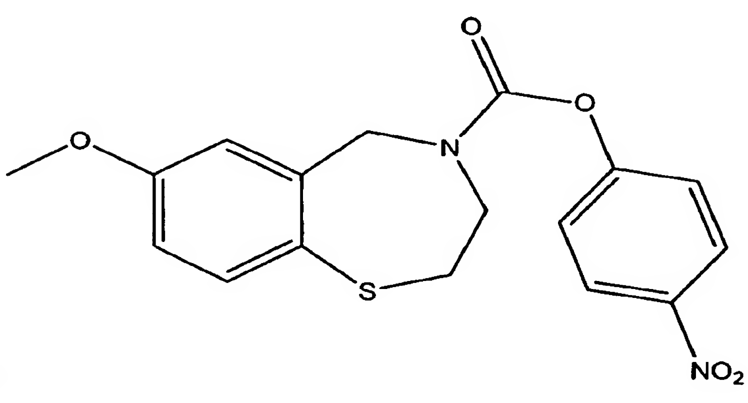
S26



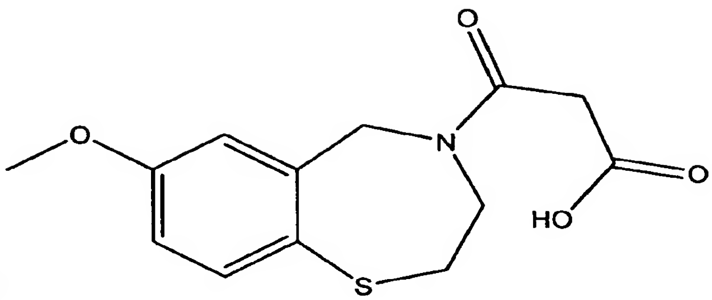
S27

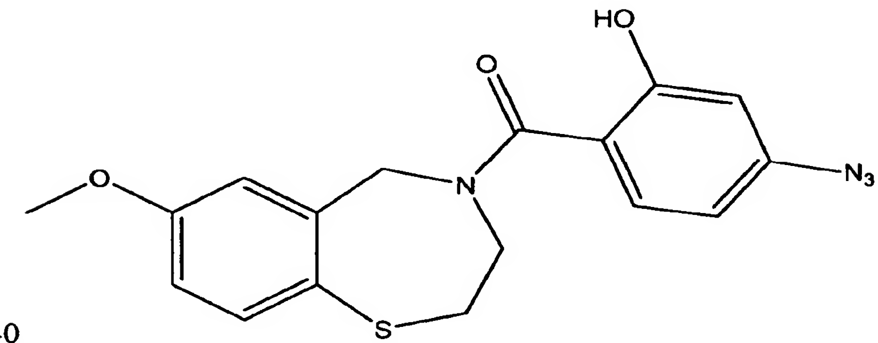


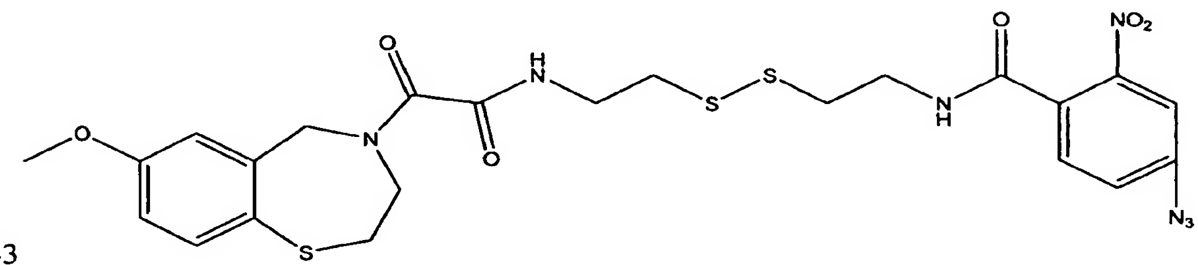
S36

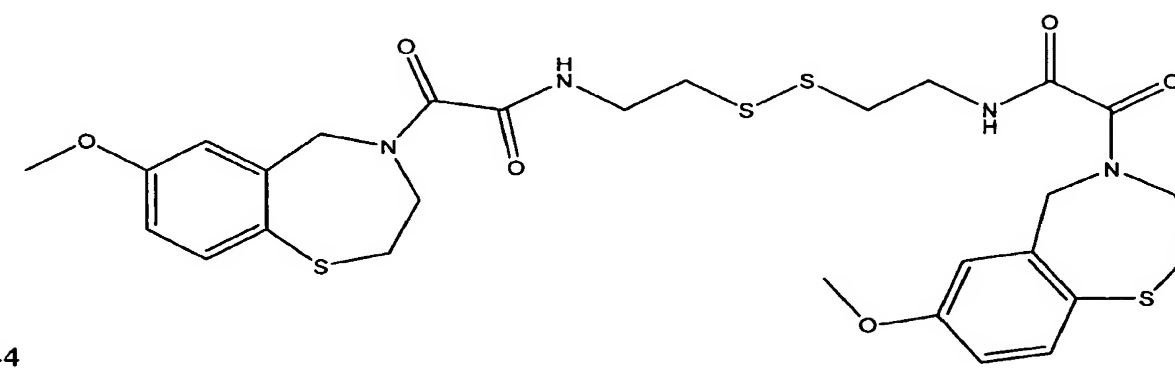


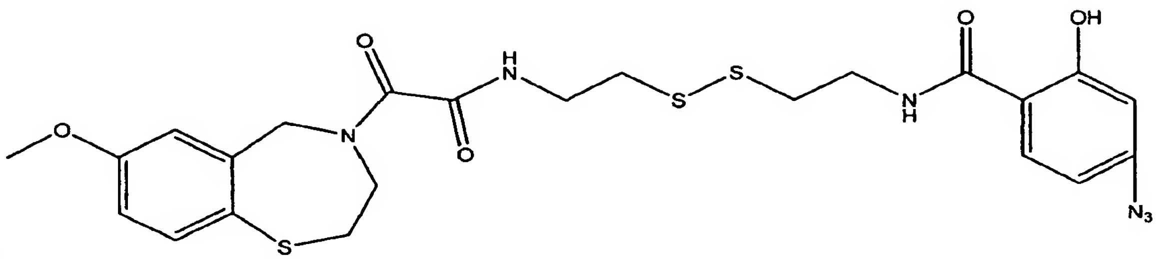
S37


S38

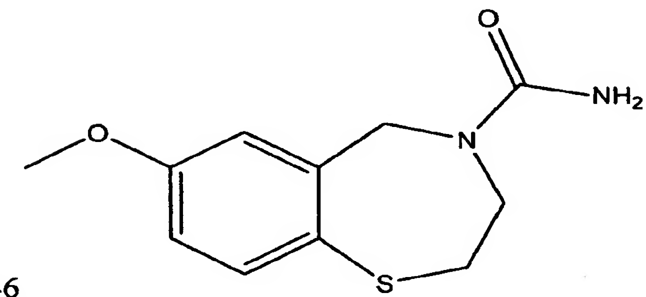

S40 ,


S43

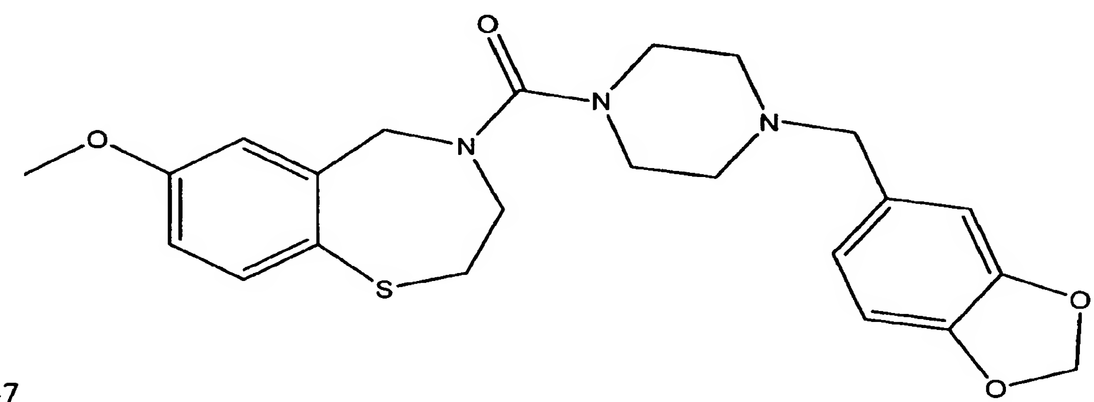

S44



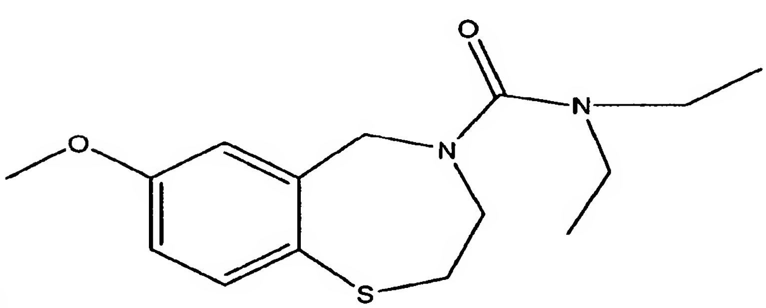
S45



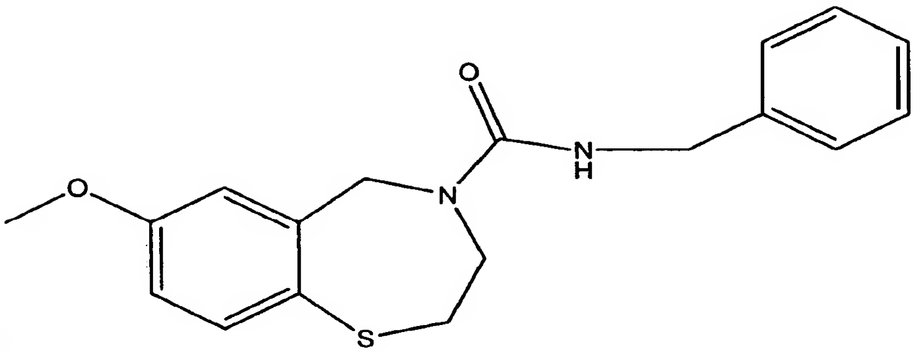
S46



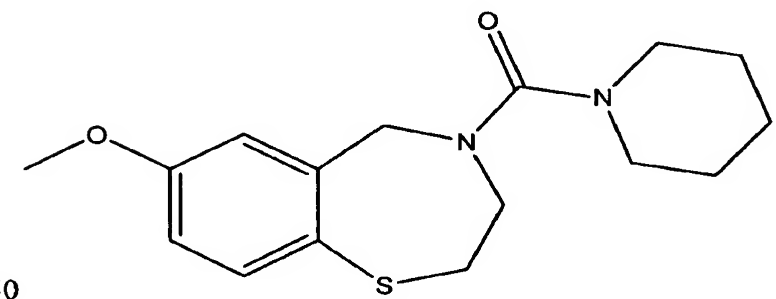
S47



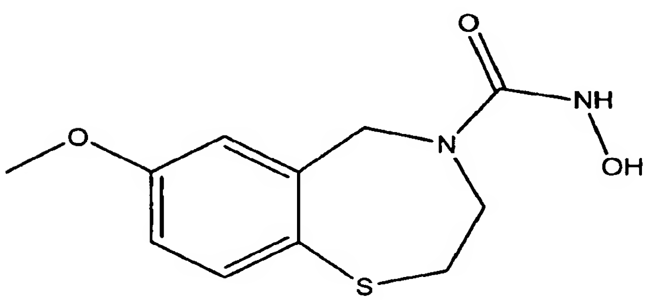
S48



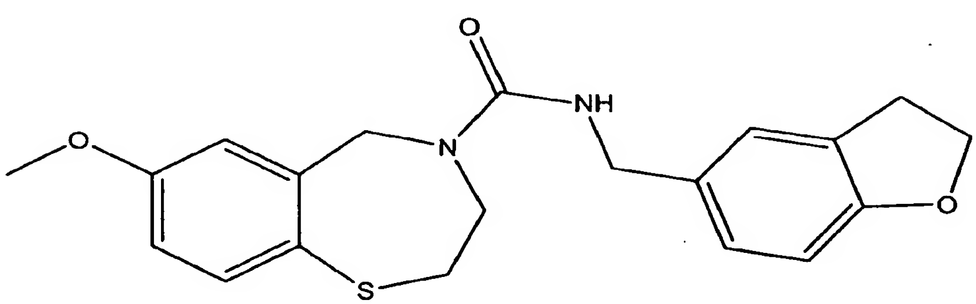
S49



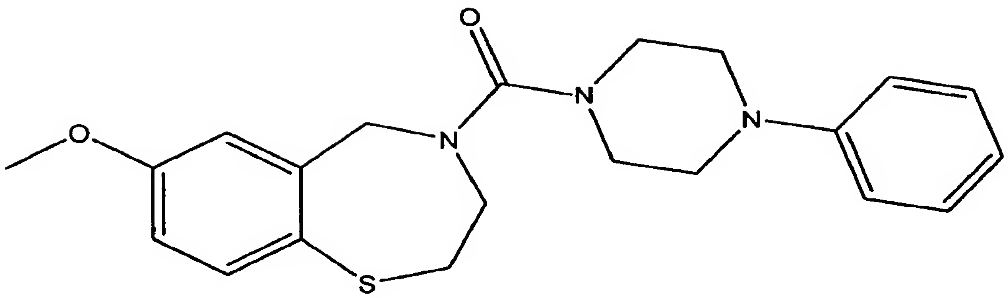
S50



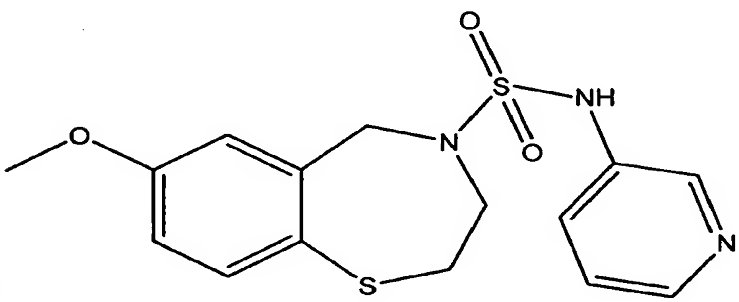
S51



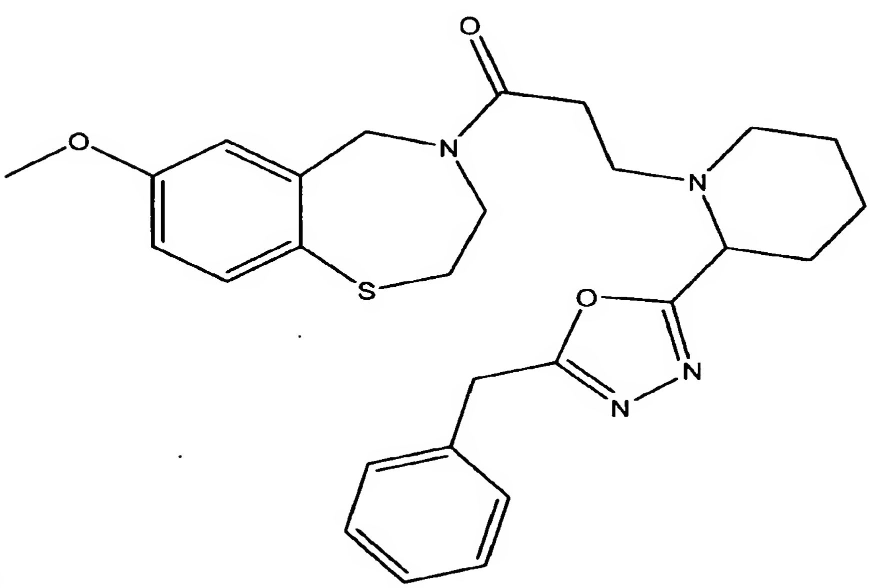
S52



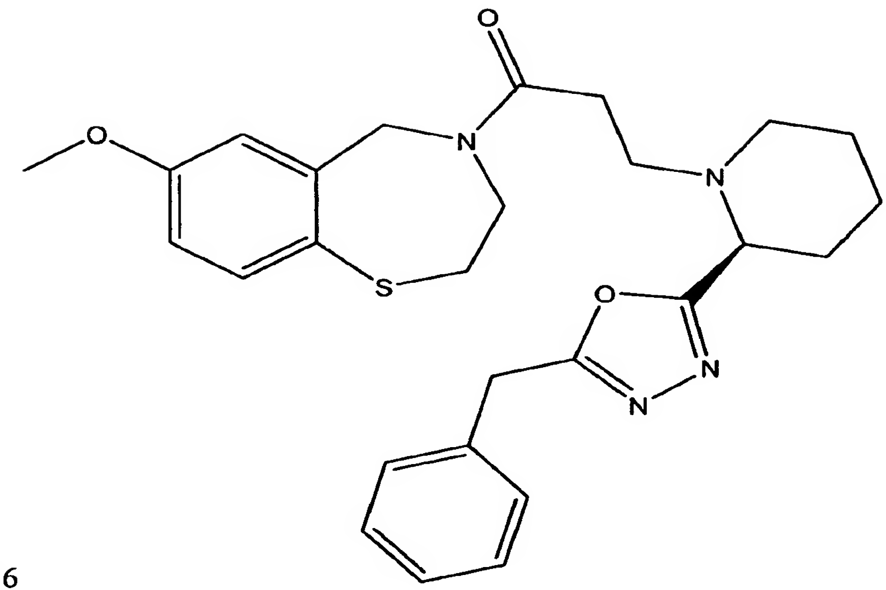
S53



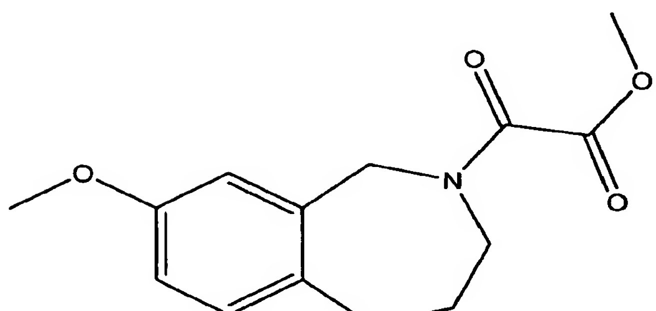
S54



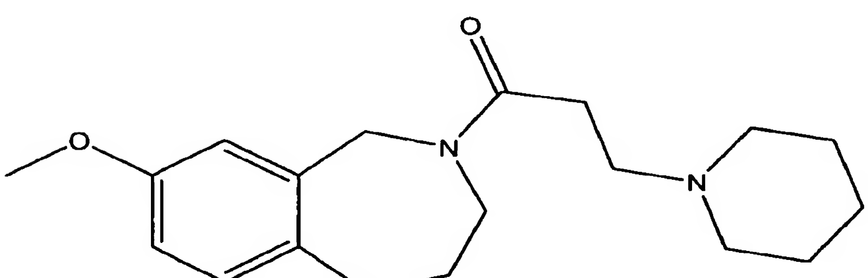
S55



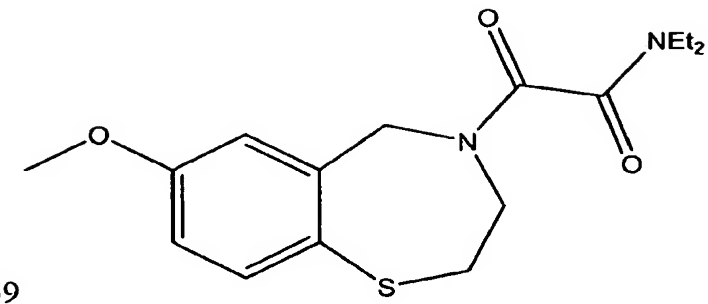
S56



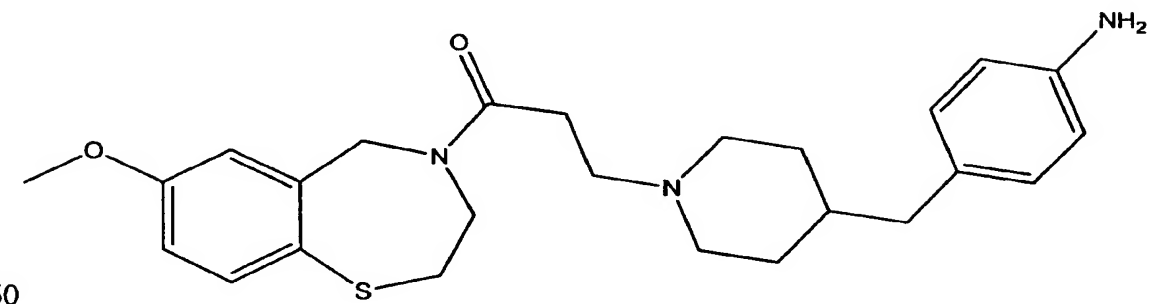
S57



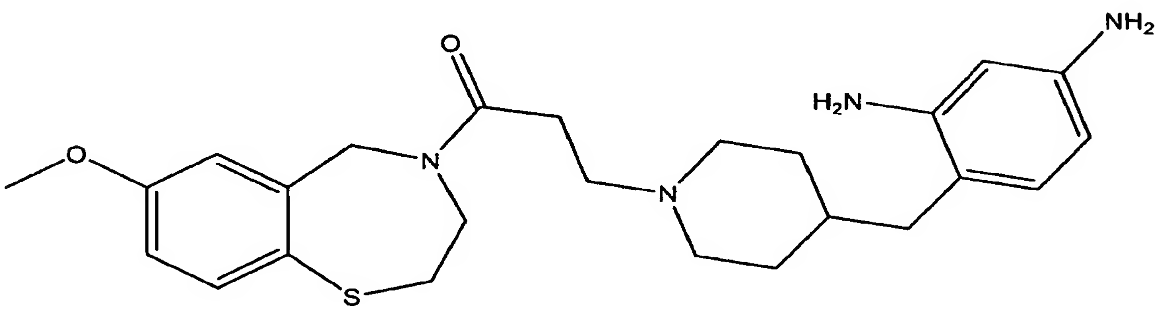
S58



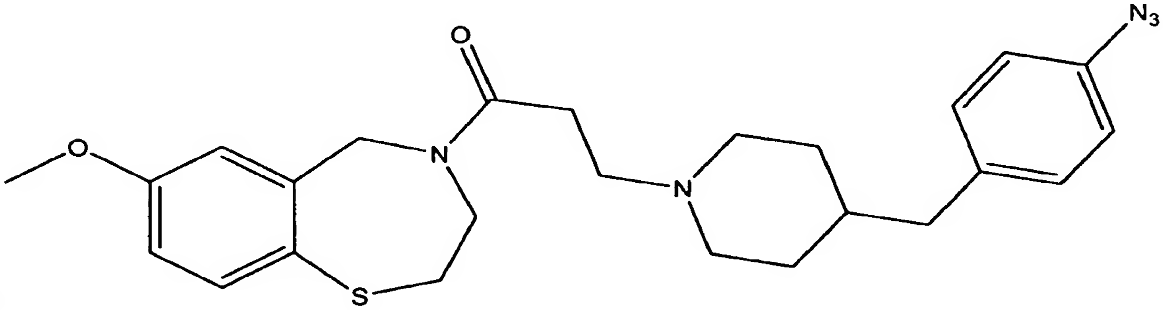
S59



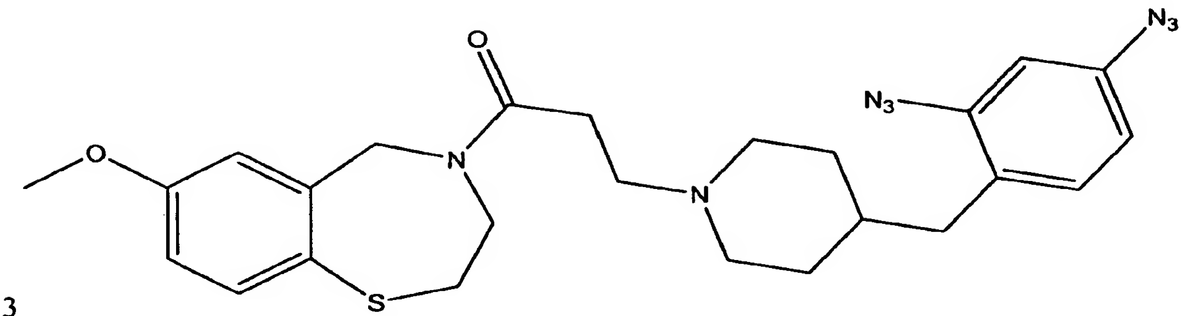
S60



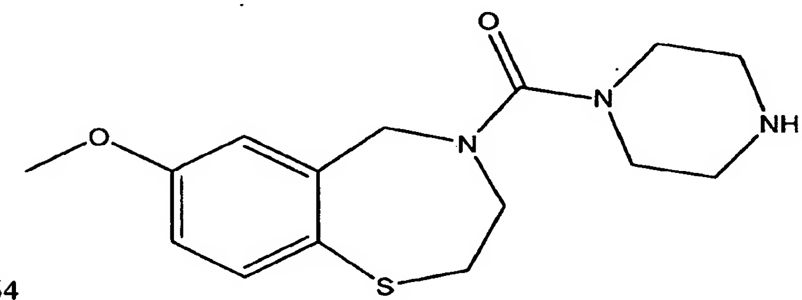
S61



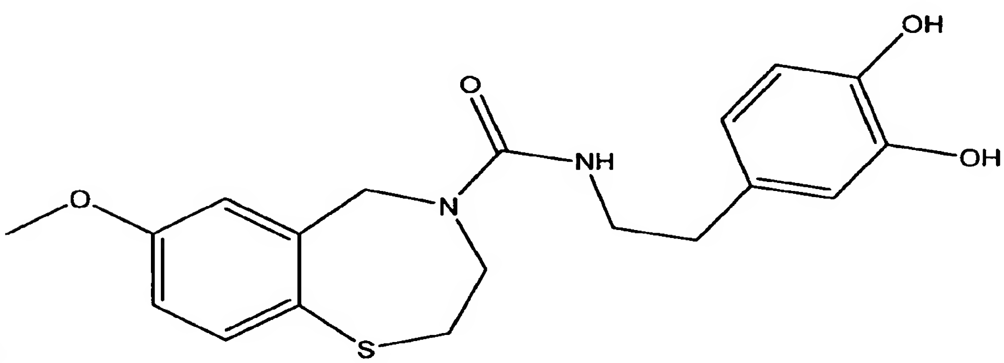
S62



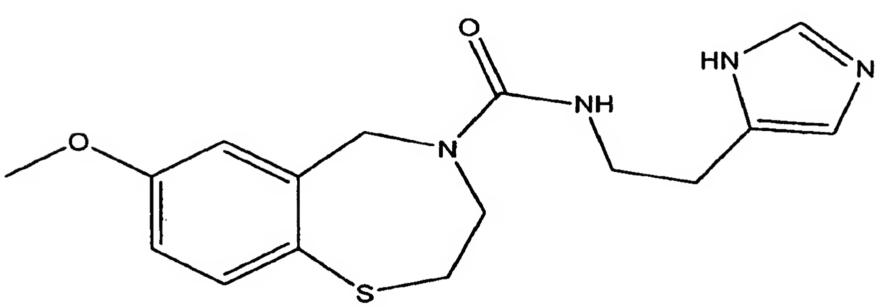
S63



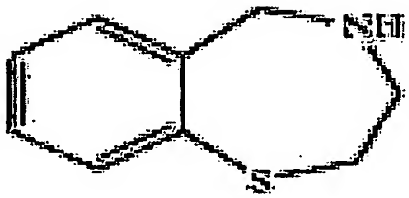
S64



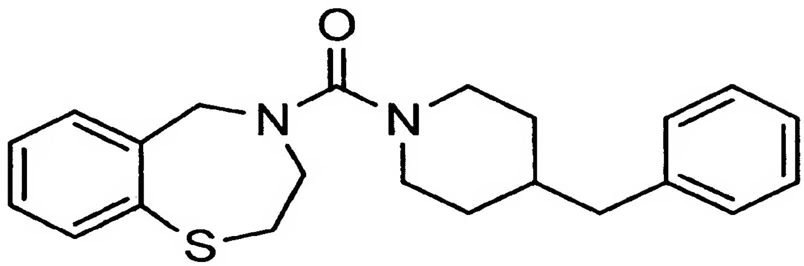
S66



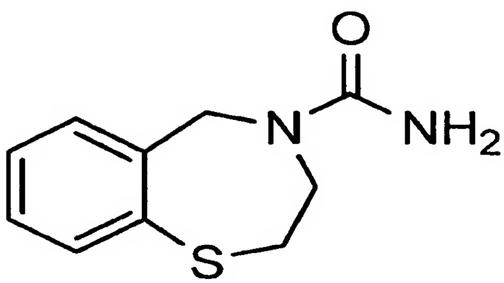
S67



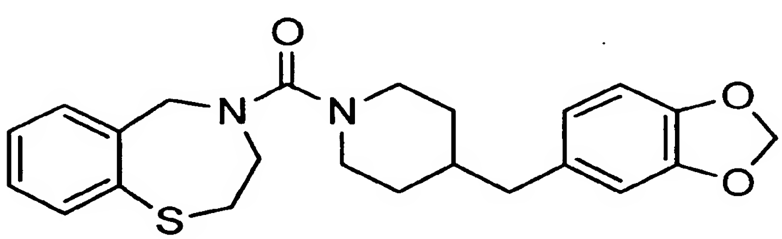
S68



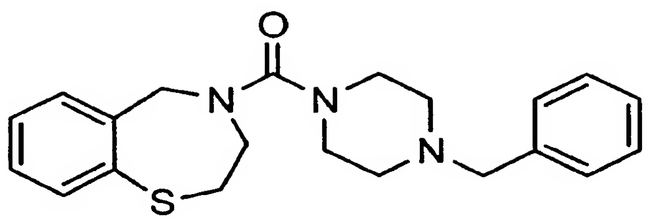
S69



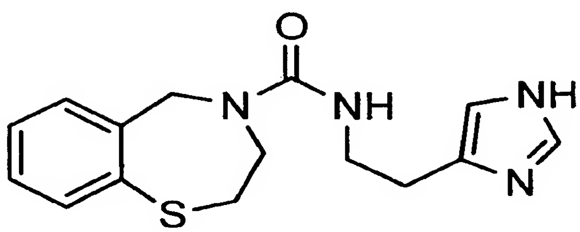
S70



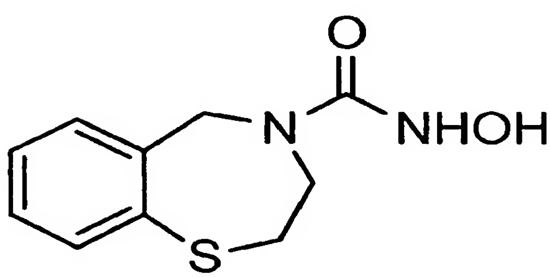
S71



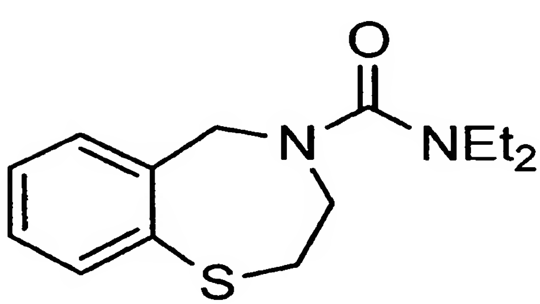
S72



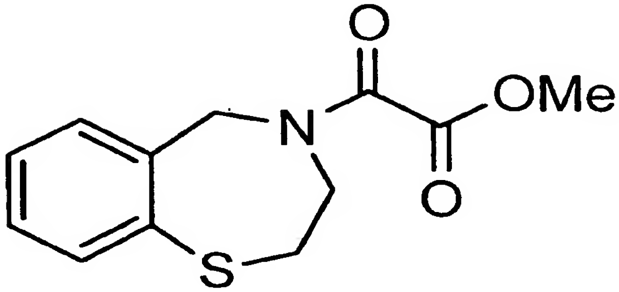
S73



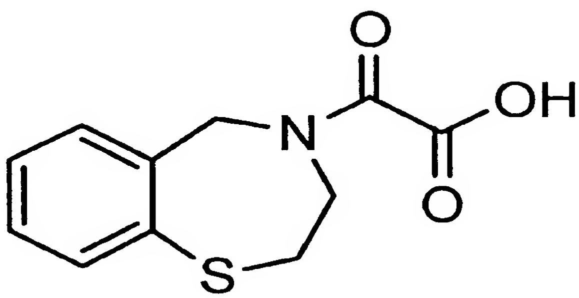
S74



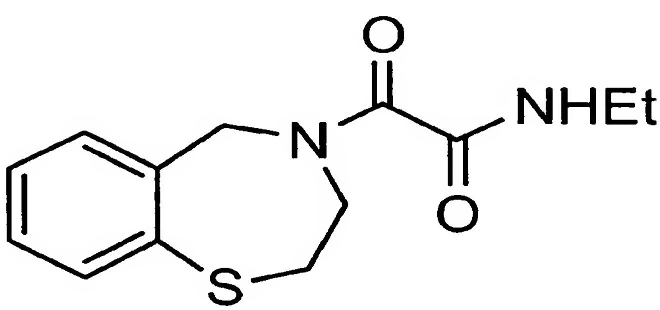
S75



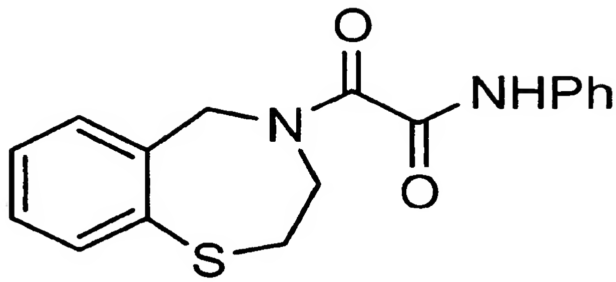
S76



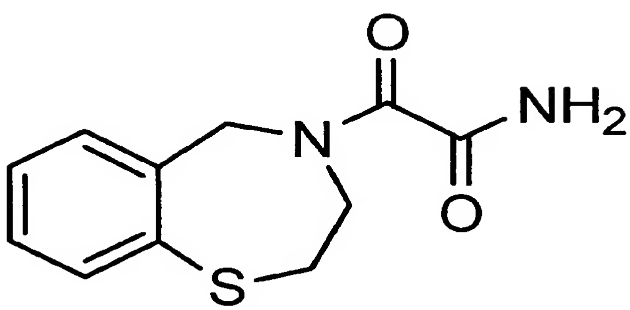
S77



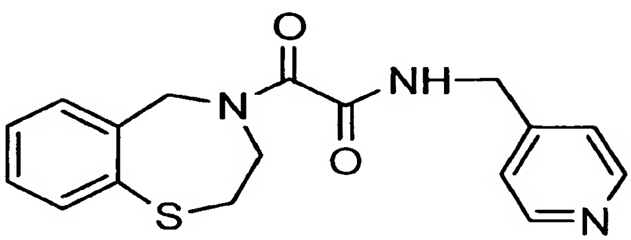
S78



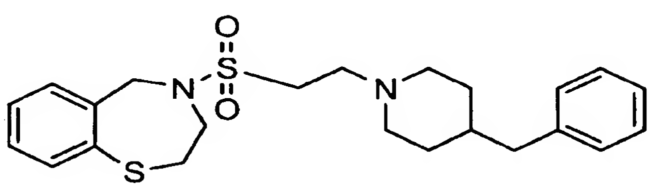
S79



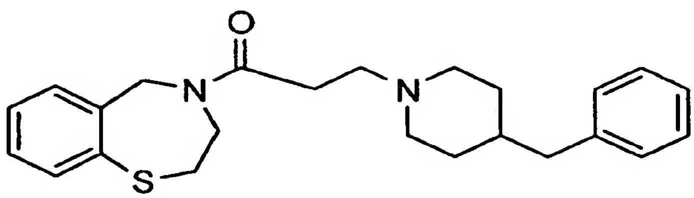
S80



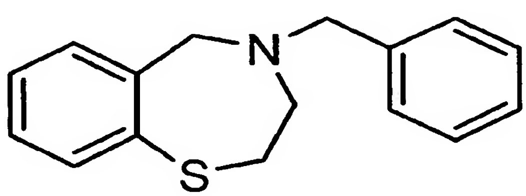
S81



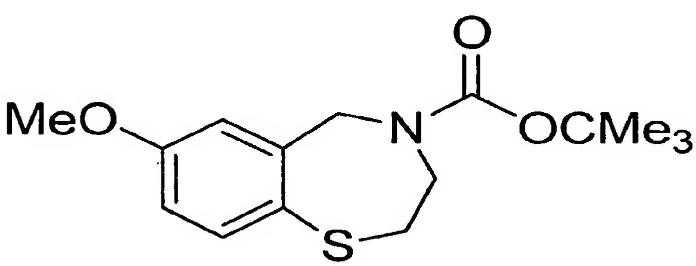
S82



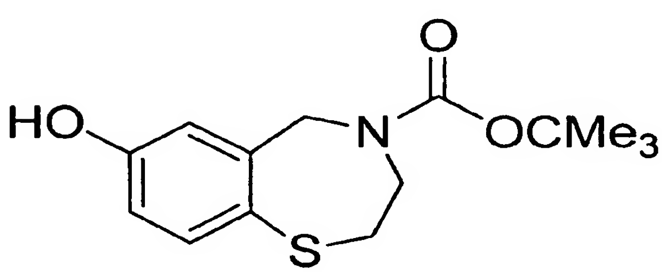
S83



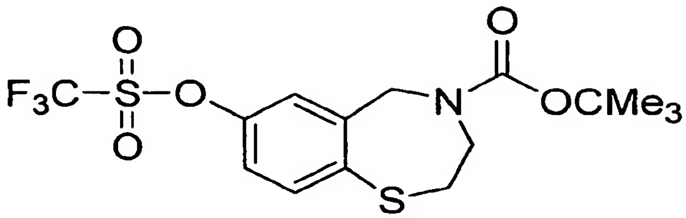
S84



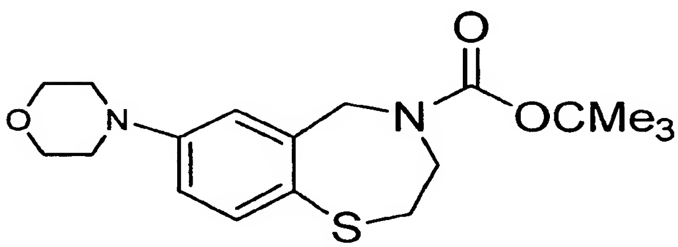
S85



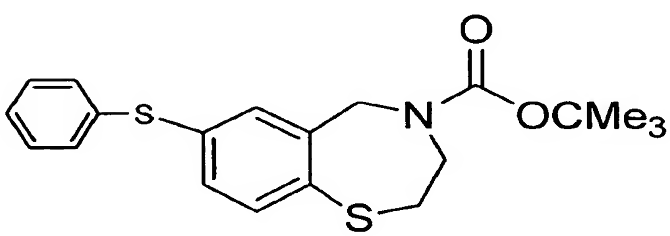
S86



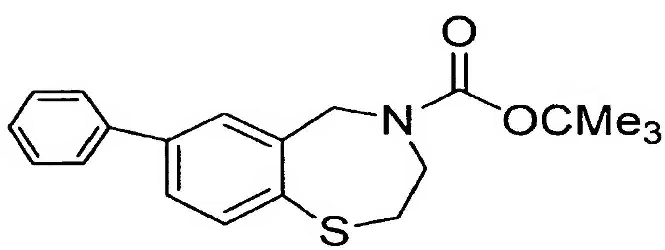
S87



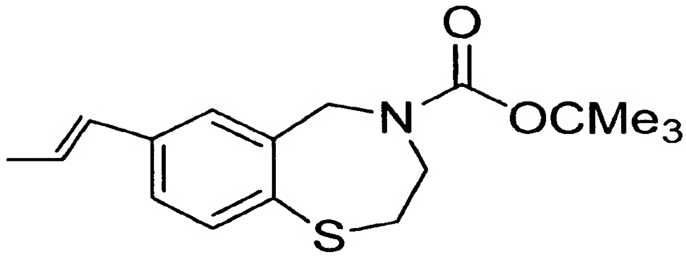
S88



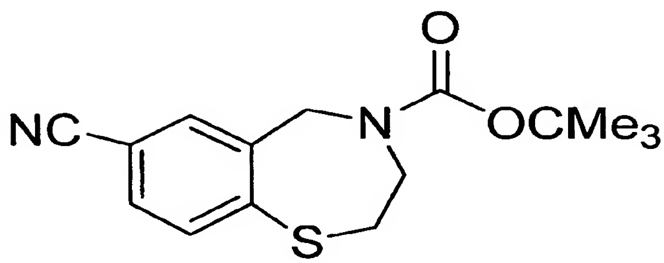
S89



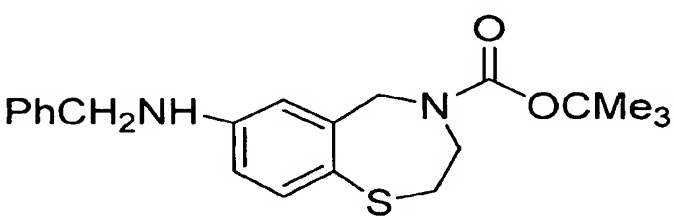
S90



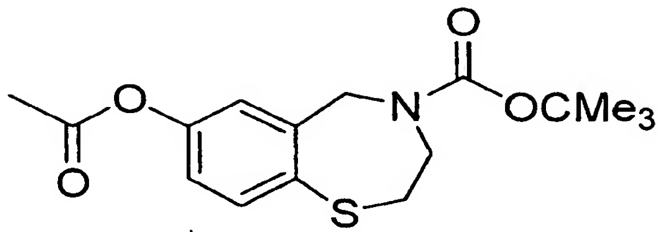
S91

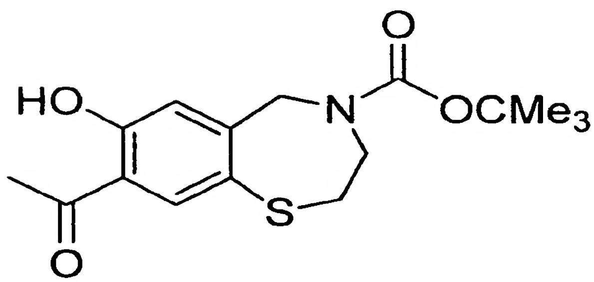


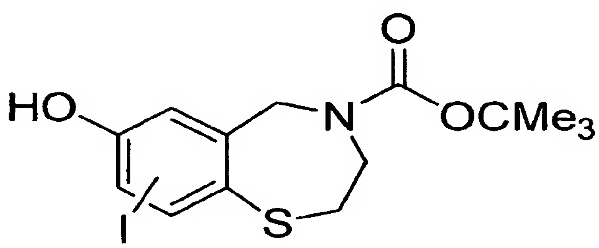
S92

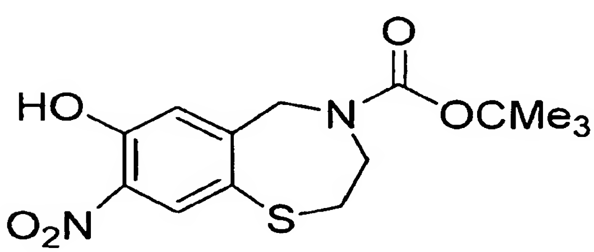


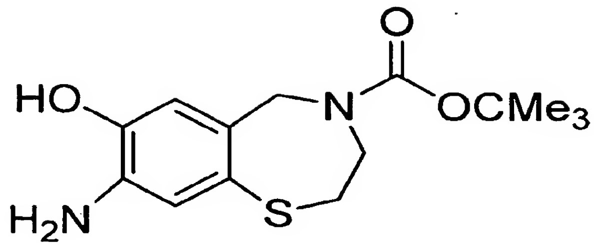
S93


S94

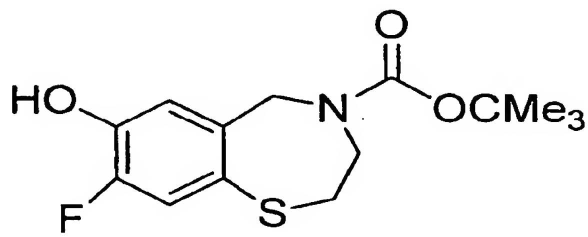

S95


S96

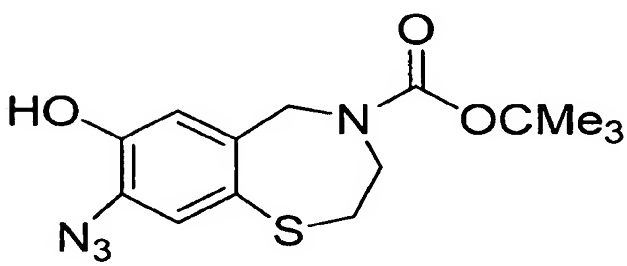

S97



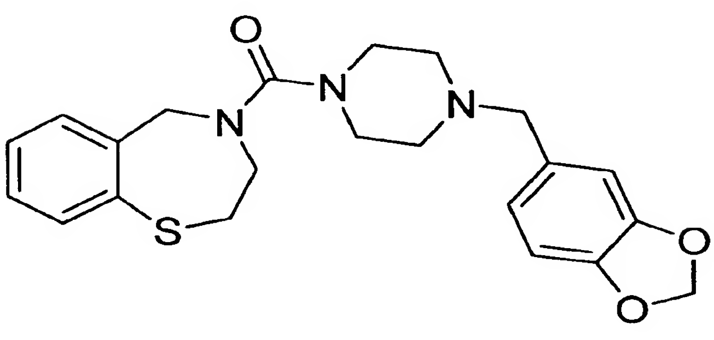
S98



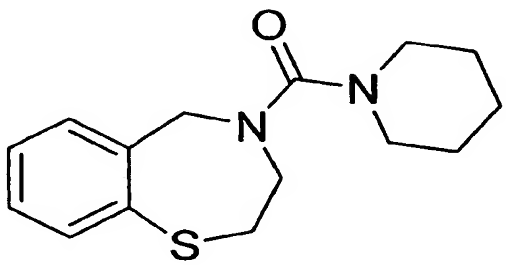
S99



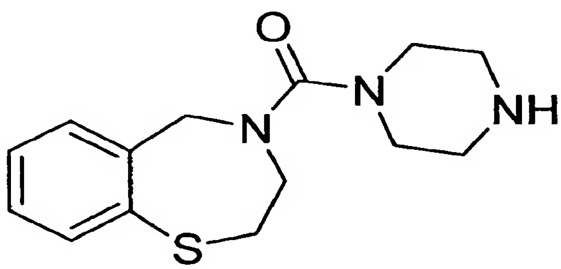
S100



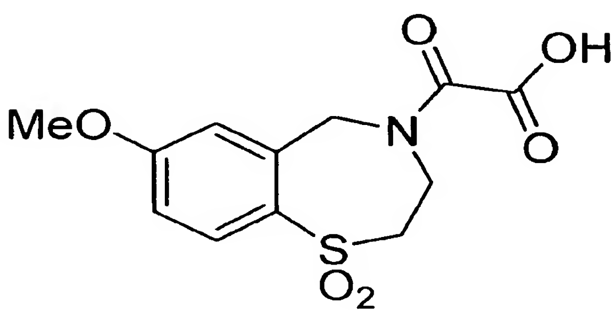
S101



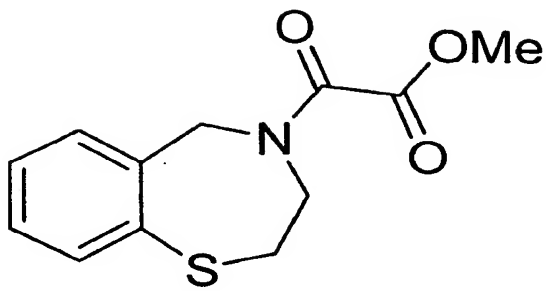
S102



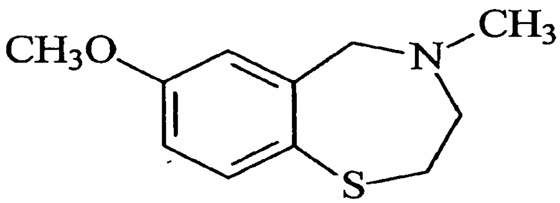
S103



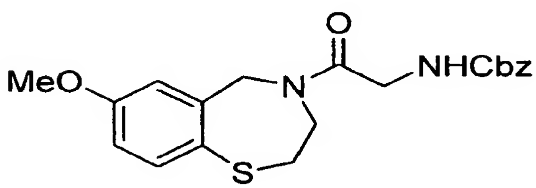
S104



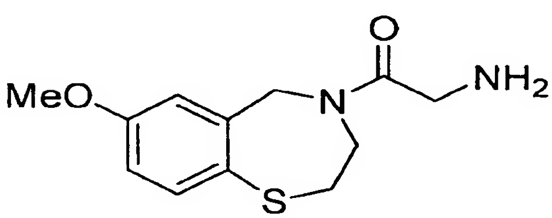
S105



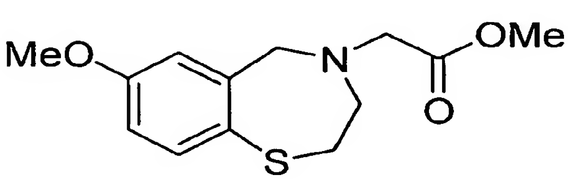
S107



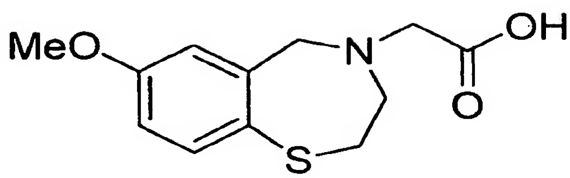
S108



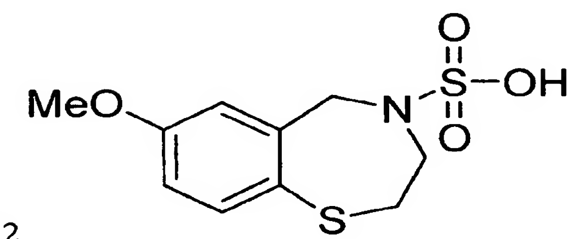
S109



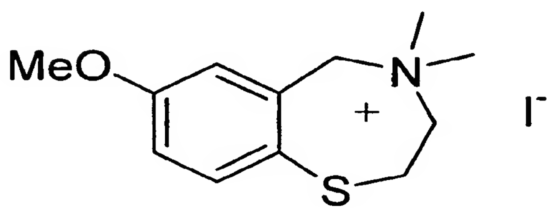
S110



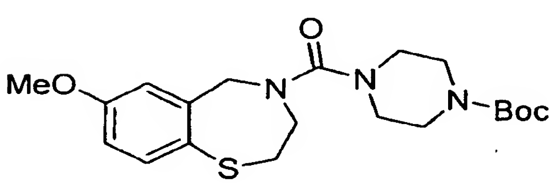
S111



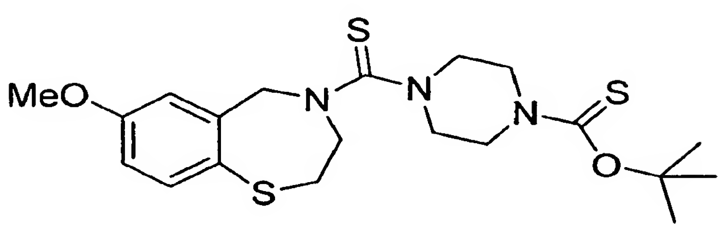
S112



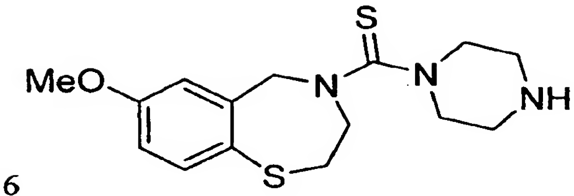
S113



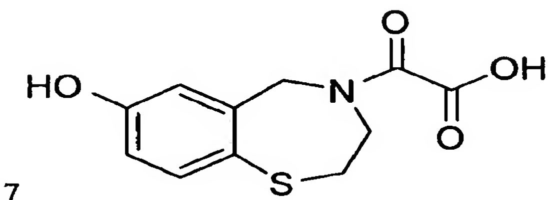
S114



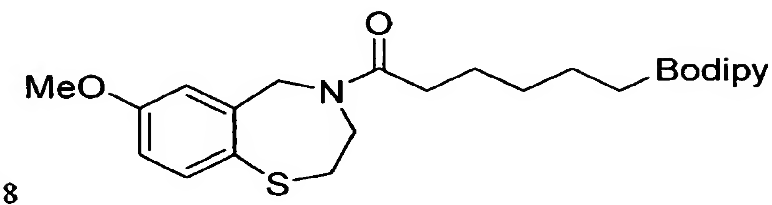
S115



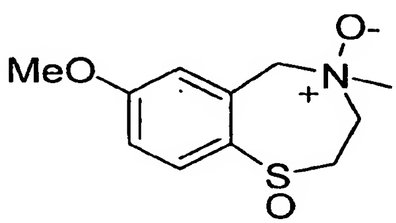
S116



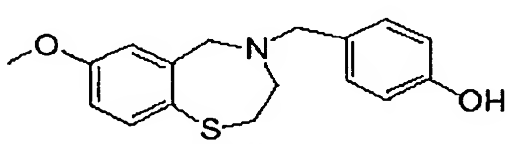
S117



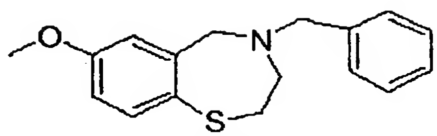
S118



S119

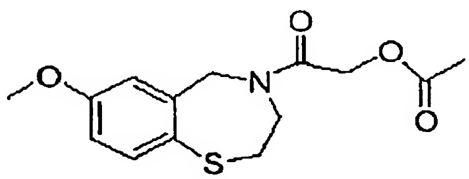


S120



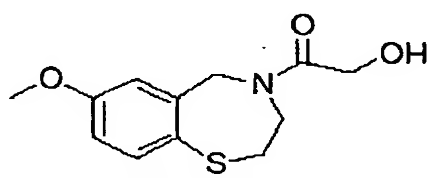
Arm121

S121



Arm122

S122



Arm123

S123

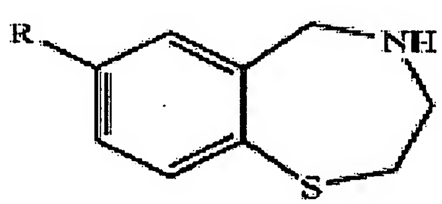
[00165] In one embodiment of the present invention, for compounds of Formula I, if R_2 is $C{=}O(R_5)$ or SO_2R_7, then R is at positions 2, 3, or 5 on the benzene ring.

[00166] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C{=}O(R_5)$ or SO_2R_7, then each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-N_3$, $-SO_3H$, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00167] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C{=}O(R_5)$ or SO_2R_7, then there are at least two R groups attached to the benzene ring. Furthermore, there are at least two R groups attached to the benzene ring, and both R groups are attached at positions 2, 3, or 5 on the benzene ring. Still furthermore, each R is independently selected from the group consisting of H, halogen, -OH, $-NH_2$, $-NO_2$, -CN, $-N_3$, $-SO_3H$, acyl, alkyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino; wherein each acyl, alkyl, alkoxyl, alkylamino, cycloalkyl, heterocyclyl, heterocyclylalkyl, alkenyl, (hetero-)aryl, (hetero-)arylthio, and (hetero-)arylamino may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, -SH, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00168] In another embodiment of the invention, for compounds of Formula I, if R_2 is $C{=}O(R_5)$, then R_5 is selected from the group consisting of $-NR_{16}$, $-(CH_2)_zNR_{15}R_{16}$, $NHNHR_{16}$, NHOH, $-OR_{15}$, $CONH_2NHR_{16}$, $CONR_{16}$, CH_2X, acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl; wherein each acyl, aryl, cycloalkyl, cycloalkylalkyl, heterocyclyl, and heterocyclylalkyl may be substituted with one or more radicals independently selected from the group consisting of halogen, N, O, -S-, -CN, $-N_3$, nitro, oxo, acyl, alkyl, alkoxyl, alkylamino, alkenyl, aryl, (hetero-)cycloalkyl, and (hetero-)cyclyl.

[00169] In another embodiment, the present invention provides use of compounds of Formula II:



wherein R=OR', SR', NR', alkyl, or halide and R' = alkyl, aryl, or H, and wherein R can be at position 6, 7, 8, or 9. Formula II is discussed also in co-pending application 10/680,988, the disclosure of which is incorporated herein in its entirety by reference.

Routes of Activity

[00170] The compounds of the invention, such as the compounds of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, reduce the open probability of RyR channels and decrease the calcium current through such channels by increasing binding of calstabin (FKBP12 or calstabin, and FKBP12.6 or calstabin2) binding affinity. Therefore, the compounds of the invention are useful for the treatment and/or prevention of disorders and conditions associated with abnormal function of RyR receptors, particularly RyR1 and RyR2 receptors, where such disorders and conditions are characterized by an increase in the open probability of, and in increase in the calcium current through, RyR receptor channels.

[00171] The present invention involves the discovery that mutations in the RyR2 channel in individuals with hypertrophic cardiomyopathy result in an increased open probability of the RyR2 channel or a "leakiness" of the RyR2 channel, and that this appears to be a causative factor in the development of cardiac hypertophy. Thus, the compounds of the invention may be useful for the treatment and/or prevention of hypertrophic cardiomyopathies caused by mutations in the RyR2 gene, and indeed other hypertrophic cardiomyopathies and other forms of cardiac hypertrophy, regardless of etiology.

[00172] In accordance with the methods of the present invention, a "decrease" or "disorder" in the level of RyR-bound FKBP in cells of a subject refers to a detectable decrease, diminution or reduction in the level of RyR-bound FKBP in cells of the subject. Such a decrease is limited or prevented in cells of a subject when the decrease is in any way halted, hindered, impeded, obstructed or reduced by the administration of compounds of the invention, such that the level of RyR-bound FKBP in cells of the subject is higher than it would otherwise be in the absence of the administered compound.

[00173] The level of RyR-bound FKBP in a subject is detected by standard assays and techniques, including those readily determined from the known art (*e.g.*, immunological techniques, hybridization analysis, immunoprecipitation, Western-blot analysis, fluorescence imaging techniques and/or radiation detection, etc.), as well as any assays and detection methods disclosed herein. For example, protein is isolated and purified from cells of a subject using standard methods known in the art, including, without limitation, extraction from the cells (*e.g.*, with a detergent that solubilizes the protein) where necessary, followed

by affinity purification on a column, chromatography (*e.g.*, FTLC and HPLC), immunoprecipitation (with an antibody), and precipitation (*e.g.*, with isopropanol and a reagent such as Trizol). Isolation and purification of the protein is followed by electrophoresis (*e.g.*, on an SDS-polyacrylamide gel). A decrease in the level of RyR-bound FKBP in a subject, or the limiting or prevention thereof, is determined by comparing the amount of RyR-bound FKBP detected prior to the administration of JTV-519 or a compound of Formula I, I-a, I-b, I-c, I-d, I-e, I-f, I-g, I-h, I-i, I-j, I-k, I-l, I-m, I-n, I-o, I-p, or Formula II, (in accordance with methods described below) with the amount detected a suitable time after administration of the compound.

[00174] A decrease in the level of RyR-bound FKBP in cells of a subject is limited or prevented, for example, by inhibiting dissociation of FKBP and RyR in cells of the subject; by increasing binding between FKBP and RyR in cells of the subject; or by stabilizing the RyR-FKBP complex in cells of a subject. As used herein, the term "inhibiting dissociation" includes blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an FKBP subunit from an RyR molecule in cells of the subject, and blocking, decreasing, inhibiting, limiting or preventing the physical dissociation or separation of an RyR molecule from an FKBP subunit in cells of the subject. As further used herein, the term "increasing binding" includes enhancing, increasing, or improving the ability of phosphorylated RyR to associate physically with FKBP (*e.g.*, binding of approximately two fold or, approximately five fold, above the background binding of a negative control) in cells of the subject and enhancing, increasing or improving the ability of FKBP to associate physically with phosphorylated RyR (*e.g.*, binding of approximately two fold, or, approximately five fold, above the background binding of a negative control) in cells of the subject. Additionally, a decrease in the level of RyR-bound FKBP in cells of a subject is limited or prevented by directly decreasing the level of phosphorylated RyR in cells of the subject or by indirectly decreasing the level of phosphorylated RyR in the cells (*e.g.*, by targeting an enzyme (such as PKA) or another endogenous molecule that regulates or modulates the functions or levels of phosphorylated RyR in the cells). In one embodiment, the level of phosphorylated RyR in the cells is decreased by at least 10% in the method of the present invention. In another embodiment, the level of phosphorylated RyR is decreased by at least 20%.

Methods of Synthesis

[00175] The compounds of the present invention may be synthesized as described in published PCT application WO 07/024717 and U.S. patent application 11/506,285, the contents of which are hereby incorporated by reference.

EXAMPLES

EXAMPLE 1 – EFFICACY OF COMPOUNDS

[00176] The compounds described herein increase binding of FKBP12 or calstabin to RyRs. Table 1 below provides EC_{50} values for compounds S1-S107. These EC_{50} data were obtained using an FKBP12.6 rebinding assay to determine the amount of FKBP12.6 binding to PKA-phosphorylated RyR2 at various concentrations (0.5 – 1000 nM) of the compounds shown in Table 1. The EC_{50} values were calculated using Michaelis-Menten curve fitting. Further details of the efficacy of these compounds, and the methods used to assess their efficacy, can be found in published PCT application WO 07/024717 and U.S. patent application 11/506,285 (US 2007/173482), the contents of which are hereby incorporated by reference.

Accordingly, the compounds of the invention may be useful for treating cardiac hypertrophy, such as cardiac hypertrophy that is associated with mutations in the RyR2 channel that result in decreased binding of calstabin2 to RyR2. The effects of such mutations on RyR2 functionining and the calstabin interaction is illustrated in. e.g. U.S. patent application 11/506,285 (US 2007/173482), the contents of which are incorporated by reference herein.

EXAMPLE 2 – CARDIAC HYPERTROPHY

[00177] Cardiac output is increased by stress-induced stimulation of β-adrenergic receptors (β-AR) which activates RyR2 via cAMP-dependent protein kinase A (PKA) phosphorylation at RyR2-S2808 (Marx et al., 2000; Wehrens et al., 2006). PKA phosphorylation of RyR2 is tightly regulated by PKA, a phosphodiesterase (PDE4D3) and phosphatases (PP1 and PP2A) bound to the RyR2 macromolecular complex via targeting proteins and leucine-isoleucine (LIZ) binding sites (Lehnart et al., 2005; Marx et al., 2001). In vivo PKA phosphorylation dynamically decreases calstabin2 binding to RyR2, resulting in increased sensitivity of Ca^{2+}-dependent activation and higher RyR2 open probability (Marx et al., 2000; Wehrens et al., 2003). As a net result, β-AR signaling increases the gain of EC coupling and cardiac output through PKA-dependent phosphorylation of RyR2 as part of a evolutionarily conserved stress pathway also known as the "fight-or-flight" response (Marks et al., 2002).

[00178] It has previously been shown that calstabin2 deficiency results in a CPVT phenotype in mice, and exercised-induced arrhythmias in the structurally normal heart (Wehrens et al., 2003). In addition, it has been shown that the missense mutations S2246L, R2474S, and R4497C identified in CPVT patients all caused a similar RyR2 gain-of-function defect during PKA-dependent stimulation and a significantly decreased calstabin2 binding affinity (Wehrens et al., 2003). Stress-testing of calstabin2 deficient mice or cardiomyocytes resulted in ventricular arrhythmias and SCD, and delayed after depolarizations (DADs) from a transient inward current (*I*ti) (Lehnart et al., 2006; Wehrens et al., 2003). It was not known whether *RyR2* mutations might exist that cause a distinct allelic disease: CPVT combined with structural heart disease, as suggested earlier (Tiso et al., 2001). The present inventors sought to characterize RyR2 mutants linked HCM in terms of their effect on RyR2 channel structure and function, and to compare these to previously characterized CPVT-mutant RyR2.

RESULTS

Genotype-phenotype correlation in RyR2 mutation carriers

[00179] The frequency of RyR2 mutations has been determined in 62 unrelated HCM probands (Fujino et al., 2006). All probands had a left ventricular wall thickness (LVWT) greater than 13 mm. Thirty-four HCM probands were excluded from further study due to mutations in sarcomeric genes (β-cardiac myosin heavy chain, cardiac troponins I and T, α-

tropomyosin, cardiac myosin binding protein C, cardiac actin and cardiac myosin essential and regulatory light chains) (Fujino et al., 2006). Four of the remaining HCM probands showed RyR2 sequence variations consistent with missense mutations in conserved residues which did not occur in 430 unrelated control subjects. The RyR2-E3654D missense mutation was found in the index subject and 4 members of family A (Table 1). The index subject showed HCM with left ventricular outflow tract obstruction and cardiac arrest from ventricular fibrillation while walking down a street at age 47 years has been witnessed. Family A has a history of sudden death, syncope, and HCM involving several siblings. The index subject of family B showed slow ventricular tachycardia and was positive for the RyR2-R929C mutation which was found in three additional family members. The family history includes sudden death during sleep and atypical chest pain. The RyR2-G2367R mutation occurred in the index subject of family C who showed marked left ventricular hypertrophy and systolic obliteration of the cavity. A DNA sample from the index case of family D with biventricular hypertrophy and outflow tract obstruction, demonstrated a positive RyR2-R2642K carrier status. An additional mutation (deletion C837) in the cardiac myosin binding protein C (MyBP-C) found in the index subject causes a relatively mild disease phenotype in other individuals. Thus, the index subject of family D has compound heterozygous mutations of RyR2- R2642K and MyBP-C-delC837.

Table 1

Phenotype of RyR2 mutation carriers with HCM and number of identified mutation carriers.

RyR2 mutations in patient with HCM	Mutation Carriers (n)	Specific changes (a)	Arrhythmias (b)	SCDs (c)
RyR2-E3654D (family A)	HCM (4)	ASH (2) LVOTO (3)	VF (1) Inducible VT (1)	Resuscitated (1) Syncope (1) Unknown (1)
RyR2-R929C (family B)	HCM (1)	ASH (1)	Slow VT (1) VT/Bradycardia (1)	During sleep (1)
RyR2-V4653F (family C)	HCM (1)	DCO (1)	none	none
RyR2-R2642K and MyBP-C-delC837 (family D)	HCM (1)	Biventricular HCM (1) LVOTO (1)	QTc 455 ms	none
All HCM RyR2 mutation carriers	HCM (7)	ASH (3) LVOTO (3)	VT, VF	SCD, Syncope

Key to Table 1

(a) Number of RyR2 mutations carriers who showed structural and/or functional cardiac abnormalities: ASH, assymetric septal hypertrophy; LVOTO, left ventricular outflow tract obstruction; DCO, distal cavity oblitaration.

(b) VF, ventricular fibrillation.

(c) Number of sudden cardiac deaths (SCDs), syncopal events, and associated findings within HCM mutation carriers.

MyBP-C, cardiac myosin binding protein C.

[00180] None of the RyR2 index mutation carriers had coronary artery disease and no structural abnormalities of the heart indicative of ARVC were found (Tiso et al., 2001).

[00181] RyR2 missense mutations linked to HCM cause a gain-of-function defect in the absence of PKA phosphorylation

[00182] To determine the functional effects of the RyR2 mutations, homo-tetrameric wild-type (WT) or HCM-mutant (RyR2-R929C, RyR2-G2367R, RyR2-R2642K, RyR2-

E3654D) and CPVT-mutant (RyR2-S2246L) channels were coexpressed with calstabin2 (FKBP12.6) (Fig. 1). All HCM-mutant RyR2 channels were phosphorylated to the same degree as WT channels by PKA *in vitro* as evidenced by RyR2-S2808 phospho-epitope specific immunoblotting (Fig. 1). PKA phosphorylation resulted in complete dissociation of calstabin2 from HCM-mutant and WT RyR2, and was specifically prevented by the PKA inhibitory peptide PKI_{5-24} (Fig. 1)(Marx et al., 2000). Under nonphosphorylated conditions, HCM-mutant RyR2 channels showed significantly decreased binding of radiolabeled calstabin2 (Fig. 2). The K_D of homo-tetrameric, HCM-mutant RyR2 channels was significantly increased compared to WT at similar B_{max} values indicating that the HCM missense mutations result in significantly decreased calstabin2 binding affinities for RyR2. Since *in vitro* PKA phosphorylation was shown to functionally dissociate calstabin2 (Fig. 1), a reduced calstabin2 binding affinity of HCM-mutant RyR2 is likely to promote abnormal calstabin2 depletion compromising the channel closed state during stress-induced PKA phosphorylation.

[00183] Nonphosphorylated RyR2-WT channels exhibited low open probability (Po 0.002±0.001) at 150 nM *cis* (cytosolic) Ca^{2+} as expected because RyR2 channels have to be tightly closed during diastole to allow for relaxation of the heart muscle to prevent Ca^{2+} dependent arrhythmias. In contrast, at 150 nM Ca^{2+} nonphosphorylated HCM-mutant channels showed a significantly increased open probability (RyR2-R929C Po 0.017±0.005; RyR2-G2367R Po 0.021±0.004; each $P<0.001$) (Fig. 3A). As arrhythmias in CPVT patients are characteristically triggered by exercise, the inventors simulated the effects of sympathetic activation on RyR2 by *in vitro* PKA phosphorylation of the channels. PKA phosphorylation significantly increased the open probability of all HCM-mutant RyR2 channels compared to WT as demonstrated by representative traces (Fig. 3B). HCM-mutant RyR2 channels revealed significantly longer open and shorter closed states and the distribution of opening events was shifted to higher current amplitudes as shown in the respective histograms (Fig. 3B).

<u>Differences between RyR2 missense mutations found in HCM versus CPVT patients</u>

[00184] The CPVT phenotype of stress-induced arrhythmias and SCD occurs characteristically during exercise or emotional stress (Lehnart et al., 2004; Priori et al., 2001). Since arrhythmias and SCD also occur in *RyR2* mutation carries with HCM, the inventors compared the single channel phenotype of PKA phosphorylated CPVT-mutant versus HCM-

mutant RyR2 channels. As compared to PKA phosphorylated WT channels, both HCM (R929C and G2367R) and CPVT (S2246L) mutant RyR2 channels showed a similar gain-of-function defect (Fig. 3B). The PKA phosphorylation induced defect in CPVT as reported earlier (Lehnart et al., 2004; Wehrens et al., 2003) and HCM channels supports SCD from acute stress-induced SR Ca^{2+} leak resulting in I_{ti}, DADs, and triggered arrhythmias as shown in the structurally normal heart (Lehnart et al., 2006; Liu et al., 2006). No significant differences occurred at the single channel level between PKA phosphorylated HCM versus CPVT mutant channels (Fig. 4B).

[00185] In the presence of the specific PKA inhibitor PKI_{5-24}, all PKA treated HCM mutant RyR2 channels showed a significantly increased open probability as compared to both WT and CPVT-mutant RyR2 (Fig. 3A). In agreement with earlier CPVT studies, RyR2-S2246L treated with PKA+PKI showed a small increase in open probability (0.003±0.001; n.s.). In contrast, all PKA+PKI treated HCM-mutant RyR2 channels showed a significantly increased open probability indicating chronic SR Ca^{2+} leak under nonphosphorylated conditions (Fig. 4A). As summarized in Fig. 4C, only HCM-mutant channels show both an increased open probability during non-phosphorylated and PKA phosphorylated conditions.

DISCUSSION

[00186] Missense mutations of the *RyR2* gene have been identified in patients with Hypertrophic Cardiomyopathy (HCM, this study) and patients with exercise-induced ventricular tachycardias and sudden death (CPVT) (Bauce et al., 2002; Laitinen et al., 2001; Priori et al., 2001). Since other known genetic causes of HCM (β-cardiac myosin heavy chain, cardiac troponin T, α-tropomyosin, cardiac myosin binding protein C, cardiac troponin I, cardiac actin and cardiac myosin essential and regulatory light chains) have been excluded in the mutation carriers, it was concluded that the RyR2-R929C, RyR2-G2367R and RyR2-E3654D mutations were causal. Further, the compound heterozygous mutations MyBP-C Del-C837 can be predicted to cause relatively mild HCM, and the RyR2-R2642K is considered causal for the severe HCM phenotype. Importantly, all HCM mutations studied here cause a single channel phenotype which is distinct from CPVT mutant RyR2 channels.

[00187] RyR2 channels containing the missense mutations identified in HCM patients (R929C, G2367R, R2642K, and E3654D) showed a significant gain-of-function defect both during PKA phosphorylation and in the absence of PKA phosphorylation. A gain-of-function

defect following PKA phosphorylation is consistent with earlier studies which found the same defect in mutant RyR2 channels containing mutations found in CPVT mutation carriers (Lehnart et al., 2004; Wehrens et al., 2003). In contrast to CPVT mutant RyR2, a smaller but significant defect occurred also in the absence of PKA phosphorylation in RyR2 channels harboring the missense mutations from HCM patients. The ~10-times increased open probability of HCM-mutant RyR2 when phosphorylation was specifically inhibited with the peptide PKI_{5-24} representing non-PKA phosphorylated conditions (Fig. 4) confers a sustained Ca^{2+} leak from the SR. Chronic SR Ca^{2+} leak may impact myocardial function in diastole and limit upregulation of contractile function in systole during stress adaptation. Moreover, sustained SR Ca^{2+} leak may impact upon cardiac energy metabolism due to compensatory increased SR Ca^{2+} pump activity as shown in heart failure (Meyer et al., 1998), gene dysregulation potentially contributing to hypertrophic remodeling, mitochondrial function, and apoptosis. The data implies that sympathetic modulation of HCM-mutant RyR2 channels via PKA phosphorylation would exacerbate the gain-of-function defect and the SR Ca^{2+} leak.

[00188] Decreased calstabin2 (FKBP12.6) levels in the RyR2 channel complex have recently been linked to progressive cardiac remodeling in heart failure resulting from myocardial infarction (Lehnart et al., 2005; Wehrens et al., 2005) as well as delayed after depolarizations (DADs), and exercise-induced arrhythmias and sudden cardiac death *in vivo* (Lehnart et al., 2006; Wehrens et al., 2003). Now significantly decreased calstabin2 binding affinities and a gain-of-function defect in distinct HCM-mutant RyR2 channels has been found. The net effect of all RyR2 missense mutations investigated are reduced calstabin2 (FKBP12.6) levels in the RyR2 channel complex which could result in increased destabilization of mutant RyR2 channel closed state during PKA phosphorylation and increased SR Ca^{2+} leak during diastole which in turn can activate inwardly depolarizing membrane currents, DADs, and triggered arrhythmias (Fozzard, 1992; Wehrens et al., 2003).

[00189] Previously it has been shown that PKA hyperphosphorylation of RyR2 channels occurs in failing human and animal hearts significantly increasing RyR2 activity and depleting calstabin2 from the channel complex resulting in increased SR Ca^{2+} leak (Lehnart et al., 2005; Marx et al., 2000; Yano et al., 2000). Treatment with β-adrenergic receptor blockers reverses PKA hyperphosphorylation and calstabin2 depletion in heart failure and the beneficial effects of β-blocker treatment in patients with exercise-induced arrhythmias may be related to prevention of RyR2 dysfunction (Marx et al., 2000; Reiken et

al., 2003b). It has also been shown that a significant component of structural remodeling and cardiac dysfunction in heart failure is related to chronic PKA hyperphosphorylation of a unique RyR2-S2808 site (Lehnart et al., 2005; Wehrens et al., 2006). Since any insult to the working myocardium can activate a compensatory neuroendocrine mechanism, and since increased catecholamine levels have been reported in HCM patients, it is likely that a chronic activation of the sympathetic nervous system contributes to the HCM phenotype and the defect in the HCM-mutant RyR2 channels. In fact, these studies would suggest a relationship between the extent of RyR2 PKA phosphorylation and the severity of the HCM phenotype. Future studies will have to investigate this possibility as well as the molecular mechanism which results in HCM in these specific mutation carriers as opposed to CPVT in other mutation carriers.

[00190] In summary, genotype-phenotype studies in RyR2 mutation carriers with HCM versus CPVT consistently show a distinct phenotype at the single channel level. Four structurally unrelated RyR2 missense mutations found in HCM patients exhibit a complex gain-of-function defect at the single-channel level. This defect was occurs under non-phosphorylated and PKA phosphorylated conditions indicating partial overlap with the CPVT phenotype characterized in distinct RyR2 mutations carriers as well as a previously not recognized phenotype occurring chronically in the resting heart. Chronic SR Ca^{2+} leak in the non-stimulated heart may cause a variety of detrimental effects which may significantly contribute to progressive remodeling of the HCM phenotype. Additionally, activation of a compensatory response of the sympathetic nervous system can be predicted to further worsen the HCM phenotype through increased SR Ca2+ leak in HMC-mutant RyR2 patients. Stabilization of the closed state of mutant RyR2 channels by increased calstabin2 binding may represent a novel pharmacologic principle to prevent HCM remodeling. Pharmacologic targeting of decreased calstabin2 levels in dysfunctional RyR2 channels may have broader implications ranging from genetic forms of HCM associated with a high incidence of SCD to more common forms caused by secondary mechanisms.

METHODS

Site-directed mutagenesis

[00191] Mutant RyR2 clones were constructed with QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA), with respective RyR2 fragments in pBluescript II

SK(-) vector templates. The sequences of the primers used for RyR2 mutagenesis were (codon coding for missense mutation underlined, nucleotide substitutions bold, 5'-3'orientation shown):

[00192] R929C, GCTGCCTGAACAGGAG<u>TGC</u>AATTA**T**AACTTACAAATGTCGCT **(SEQ ID NO. 1)**;

[00193] G2367R, TCCCTCACCAAATAGC<u>AGA</u>TCCAGTAAAACACTTGAC **(SEQ ID NO. 2)**;

[00194] R2642K,GCCTCAGAAGAAGAACTACATTTATCA<u>AAA</u>AAGTTGTTCTGGGGCA **(SEQ ID NO. 3)**;

[00195] E3654D,GCTGAACCTCCAGAA<u>GAC</u>GATGAAGG**T**ACCAAGAGAGTTGATCCTC **(SEQ ID NO. 4)**.

[00196] Silent nucleotide substitutions (marked bold) were introduced to facilitate restriction site screening. The fragments containing the missense mutations were sub-cloned back to their original positions in a full-length RyR2 cDNA in the pCMV5 expression vector with *BstE*II and *Acc65*I (RyR2-G2367R), *Acc65*I and *Mfe*I (RyR2-R2642K), *Nhe*I and *Fse*I (RyR2-E3654D), or by two steps with *Not*I and *Cla*I, then with *Not*I and *Acc65*I (RyR2-R929C).

<u>Ryanodine receptor expression and purification</u>

[00197] HEK293 cells grown in MEM medium supplemented with 10% (v/v) fetal bovine serum (Invitrogen, CA), penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM) were co-transfected with 20 μg of wild-type (WT) or different HCM-mutant RyR2 cDNA and 5 μg of calstabin2 (FKBP12.6) cDNA by Ca^{2+} phosphate precipitation for the expression of homotetrameric channels. Forty-eight hours after transfection heavy SR vesicles were isolated from HEK293 cell lysates. Cells were collected in 1.0 ml of PBS based lysis buffer containing 10 mM HEPES (pH 7.0), 10 mM NaF, 1.0 mM Na_3VO_4, and a protease inhibitor mixture (Complete tablets; Roche Diagnostics, Germany) and centrifuged in the same solution at 2500 g for 5 min at 4 C. After resuspending the pellet in 0.5 ml of 20 mM HEPES-NaOH, pH 7.5, containing the protease inhibitor mix, cells were lysed by passage through a 25 gauge needle 40 times. Centrifugation

of the cell homogenate diluted in ice-cold buffer containing 500 mM sucrose, pH 7.2, was performed at 10,000 g at 4°C for 15 min, followed by centrifugation of the supernatant at 100,000 g for 45 min. Protein concentration was assayed (BCA kit, Pierce Biochemical) and resuspended pellets were aliquoted and stored at -80°C for subsequent analysis.

Calstabin2 Binding Assay

[00198] $[^{35}S]$-labeled calstabin2 was generated using the TNT Quick Coupled Transcription/Translation system from Promega (Madison, WI). $[^{3}H]$ ryanodine binding was used to quantify RyR2 levels. 50 μg of microsomes were diluted in 100 μl of 10 mM imidazole buffer (pH 6.8) and incubated with 20 to 1000 nM (final concentration) $[^{35}S]$-calstabin2 at 37°C for 60 min, then quenched with 500 μl ice-cold imidazole buffer. Samples were centrifuged at 100,000 x g for 10 min, washed three times in imidazole buffer, and the amount of bound $[^{35}S]$-calstabin2 was determined by liquid scintillation counting of the pellet.

[00199] Phosphorylation and immunoprecipitation of ryanodine receptors.

[00200] Microsomes containing recombinant RyR2 isolated from transfected HEK293 cells were *in vitro* phosphorylated by the PKA catalytic subunit as described previously (Marx et al., 2000). Specificity of PKA phosphorylation was demonstrated using the specific PKA inhibitor PKI_{5-24} (Calbiochem, San Diego, CA) (Reiken et al., 2001), and quantified using the back-phosphorylation technique (Marx et al., 2000).

Immunoblots

[00201] Immunoblot analysis was performed using the primary antibodies anti-RyR (5029, 1:3000) (Brillantes et al., 1994b), anti-FKBP12 (1:1000) (Jayaraman et al., 1992), or anti-phospho-RyR2 (P2808, 1:5,000) as described previously (Reiken et al., 2003a). The P2808 phosphoepitope-specific anti-RyR2 antibody is an affinity purified polyclonal rabbit antibody custom-made by Zymed Laboratories (San Francisco, CA) using the peptide CRTRRI-(pS)-QTSQ corresponding to RyR2 PKA phosphorylated at S2808. Triplicate samples of 50 μl of purified microsomes were loaded onto SDS-PAGE gels (6% for RyR2, 15% for calstabin2). After electrophoretic separation, proteins were transferred to nitrocellulose membranes overnight (Semi-Dry transfer blot, Bio-Rad, USA) and incubated for 1 hour in TBS with 1% Tween-20 and 5% nonfat milk to prevent non-specific antibody

binding. Membranes were washed in Tween-TBS and then incubated for 60 min at room temperature with the primary antibody. After washout of the primary antibody, membranes were incubated with HRP labeled anti-rabbit IgG (1:5,000, Transduction Laboratories, Lexington, KY). Blots were washed 3 times with TBS and 0.1% Tween 20 and developed with a chemiluminescent detection system (Hyperfilm enhanced chemiluminescence, Amersham Biosciences, Piscataway, NJ). Band densities were quantified and normalized for protein concentration using Quantity One software (Biorad, Hercules, CA)(Reiken et al., 2003b).

Phosphorylation and immunoprecipitation of ryanodine receptors

[00202] RyR2 channels were immunoprecipitated from 200 μg of cell lysate homogenates with anti-RyR antibody in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.4), 0.9% NaCl, 5.0 mM NaF, 1.0 mM Na_3VO_4, 0.25% Triton-X100, and protease inhibitors overnight at 4°C. The samples were incubated with protein A sepharose beads (Amersham Biosciences, Piscataway, NJ) at 4°C for 1 hour after which the beads were washed three times with kinase buffer (8 mM $MgCl_2$, 10 mM EGTA, and 50 mM Tris/piperazine-N,N'-bis-2-ethanesulfonic acid, pH 6.8). Then RyR2 was in vitro phosphorylated by resuspending the beads in 10 μl of 1.5 x kinase buffer containing PKA catalytic subunit (Sigma-Aldrich, St. Louis, MO). RyR2 was in vitro phosphorylated by resuspending the beads in 10 μl of 1.5 x kinase buffer containing PKA catalytic subunits. The specificity of the phosphorylation was demonstrated using PKA plus the specific PKA inhibitor PKI_{5-24} (Calbiochem, San Diego, CA) (Reiken et al., 2001). Back-phosphorylation of the immunoprecipitated RyR2 was initiated with 5 μl of 100 μM Mg-ATP containing 10% [γ-^{32}P]-ATP (NEN Life Sciences, Boston, MA). The reaction was terminated after 8 min at 20°C with 5 μl of stop solution (4% SDS and 0.25 M DTT). To quantify PKA phosphorylation, non-phosphorylated and phosphorylated samples were heated to 95°C, and proteins were size fractionated on a 6% SDS PAGE gel. RyR2 radioactivity signals were quantified by densitometry using a Molecular Dynamics phosphorimager and ImageQuant software (Amersham Biosciences, Piscataway, NJ). Nonspecific phosphorylation (not inhibited by PKI-specific peptide) was subtracted, and the resulting value was divided by the amount of RyR2 protein and expressed as the inverse of the PKA-dependent ^{32}P signal (Marx et al., 2000). The specificity of the phosphorylation was demonstrated using PKA plus the specific PKA inhibitor PKI_{5-24} (Calbiochem, San Diego, CA) (Reiken et al., 2001).

Single-channel recordings

[00203] Vesicles containing RyR2 were incorporated into planar lipid bilayers in 120 μm holes of polystyrene cups separating two chambers. The trans chamber (1.0 ml) representing the intra-SR compartment was connected to the head stage input of a bilayer voltage-clamp amplifier (Warner Instruments, Hamden, CT). The cis chamber (1.0 ml) representing the cytoplasmic compartment was held at virtual ground. Symmetrical solutions used are (in mM): trans HEPES 250 and $Ba(OH)_2$ 53, pH 7.35; cis HEPES 250, Tris 125, EGTA 1.0, and $CaCl_2$ 0.5, pH 7.35. Free [Ca^{2+}] and [Mg^{2+}] were calculated by CHELATOR software. Spontaneous fusion was facilitated by increasing K^+ concentracion, after which the cis chamber was perfused with 10 ml of the original solution. To determine sensitivity to Mg^{2+}, the cis (cytoplasmic) concentration of Mg^{2+} was sequentially increased from 0.0 to 3.0 mM in 1.0 mM steps. Histogram analysis was performed for RyR2 channels activated by 1.0 μM Ca^{2+}. At the conclusion of each experiment, ryanodine (5 μM) or ruthenium red (20 μM) were applied to confirm RyR2 channel identity. Recordings were digitized at 4 kHz (Digidata 1322A; Axon Instruments, Union City, CA). Data were analyzed with Fetchan software (Axon Instruments). Data are expressed as mean ± SEM. Statistical analysis was performed with unpaired Student's t-test and a P-value <0.05 was considered significant.

References

Bauce, B., Rampazzo, A., Basso, C., Bagattin, A., Daliento, L., Tiso, N., Turrini, P., Thiene, G., Danieli, G.A., and Nava, A. (2002). Screening for ryanodine receptor type 2 mutations in families with effort-induced polymorphic ventricular arrhythmias and sudden death: early diagnosis of asymptomatic carriers. J Am Coll Cardiol *40,* 341-349.

Bers, D.M. (2002). Cardiac excitation-contraction coupling. Nature *415*, 198-205.

Brillantes, A.B., Ondrias, K., Scott, A., Kobrinsky, E., Ondriasova, E., Moschella, M.C., Jayaraman, T., Landers, M., Ehrlich, B.E., and Marks, A.R. (1994a). Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. Cell *77*, 513-523.

Brillantes, A.M., Bezprozvannaya, S., and Marks, A.R. (1994b). Developmental and tissue-specific regulation of rabbit skeletal and cardiac muscle calcium channels involved in excitation-contraction coupling. Circ Res *75*, 503-510.

Fabiato, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol *245*, C1-14.

Fozzard, H.A. (1992). Afterdepolarizations and triggered activity. Basic Res Cardiol *87 Suppl 2*, 105-113.

Fujino, N., Basson, C.T., McDonough, B., McDermitt, D.A., Seidman, C.E., and Seidman, J.G. (2006). Missense mutations in cardiac ryanodine receptor gene can cause hypertrophic cardiomyopathy. (submitted).

Jayaraman, T., Brillantes, A.M., Timerman, A.P., Fleischer, S., Erdjument-Bromage, H., Tempst, P., and Marks, A.R. (1992). FK506 binding protein associated with the calcium release channel (ryanodine receptor). J Biol Chem *267*, 9474-9477.

Laitinen, P.J., Brown, K.M., Piippo, K., Swan, H., Devaney, J.M., Brahmbhatt, B., Donarum, E.A., Marino, M., Tiso, N., Viitasalo, M., *et al.* (2001). Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. Circulation *103*, 485-490.

Leenhardt, A., Lucet, V., Denjoy, I., Grau, F., Ngoc, D.D., and Coumel, P. (1995). Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation *91*, 1512-1519.

Lehnart, S.E., Terrenoire, C., Reiken, S., Wehrens, X.H., Song, L.S., Tillman, E.J., Mancarella, S., Coromilas, J., Lederer, W.J., Kass, R.S., *et al.* (2006). Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. Proc Natl Acad Sci U S A *103*, 7906-7910.

Lehnart, S.E., Wehrens, X.H., Laitinen, P.J., Reiken, S.R., Deng, S.X., Cheng, Z., Landry, D.W., Kontula, K., Swan, H., and Marks, A.R. (2004). Sudden death in familial polymorphic ventricular tachycardia associated with calcium release channel (ryanodine receptor) leak. Circulation *109*, 3208-3214.

Lehnart, S.E., Wehrens, X.H., Reiken, S., Warrier, S., Belevych, A.E., Harvey, R.D., Richter, W., Jin, S.L., Conti, M., and Marks, A.R. (2005). Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias. Cell *123*, 25-35.

Liu, N., Colombi, B., Memmi, M., Zissimopoulos, S., Rizzi, N., Negri, S., Imbriani, M., Napolitano, C., Lai, F.A., and Priori, S.G. (2006). Arrhythmogenesis in Catecholaminergic Polymorphic Ventricular Tachycardia. Insights From a RyR2 R4496C Knock-In Mouse Model. Circ Res.

Marks, A.R., Marx, S.O., and Reiken, S. (2002). Regulation of ryanodine receptors via macromolecular complexes: a novel role for leucine/isoleucine zippers. Trends Cardiovasc Med *12*, 166-170.

Marks, A.R., Tempst, P., Hwang, K.S., Taubman, M.B., Inui, M., Chadwick, C., Fleischer, S., and Nadal-Ginard, B. (1989). Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. Proc Natl Acad Sci U S A *86*, 8683-8687.

Marx, S.O., Reiken, S., Hisamatsu, Y., Gaburjakova, M., Gaburjakova, J., Yang, Y.M., Rosemblit, N., and Marks, A.R. (2001). Phosphorylation-dependent regulation of ryanodine receptors: a novel role for leucine/isoleucine zippers. J Cell Biol *153*, 699-708.

Marx, S.O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosemblit, N., and Marks, A.R. (2000). PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. Cell *101*, 365-376.

Meyer, M., Keweloh, B., Guth, K., Holmes, J.W., Pieske, B., Lehnart, S.E., Just, H., and Hasenfuss, G. (1998). Frequency-dependence of myocardial energetics in failing human myocardium as quantified by a new method for the measurement of oxygen consumption in muscle strip preparations. J Mol Cell Cardiol *30*, 1459-1470.

Priori, S.G., Napolitano, C., Tiso, N., Memmi, M., Vignati, G., Bloise, R., Sorrentino, V., and Danieli, G.A. (2001). Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. Circulation *103*, 196-200.

Reiken, S., Gaburjakova, M., Gaburjakova, J., He Kl, K.L., Prieto, A., Becker, E., Yi Gh, G.H., Wang, J., Burkhoff, D., and Marks, A.R. (2001). beta-adrenergic receptor blockers restore cardiac calcium release channel (ryanodine receptor) structure and function in heart failure. Circulation *104*, 2843-2848.

Reiken, S., Lacampagne, A., Zhou, H., Kherani, A., Lehnart, S.E., Ward, C., Huang, F., Gaburjakova, M., Gaburjakova, J., Rosemblit, N., *et al.* (2003a). PKA phosphorylation activates the calcium release channel (ryanodine receptor) in skeletal muscle: defective regulation in heart failure. J Cell Biol *160*, 919-928.

Reiken, S., Wehrens, X.H., Vest, J.A., Barbone, A., Klotz, S., Mancini, D., Burkhoff, D., and Marks, A.R. (2003b). Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. Circulation *107*, 2459-2466.

Timerman, A.P., Jayaraman, T., Wiederrecht, G., Onoue, H., Marks, A.R., and Fleischer, S. (1994). The ryanodine receptor from canine heart sarcoplasmic reticulum is associated with a novel FK-506 binding protein. Biochem Biophys Res Commun *198*, 701-706.

Tiso, N., Stephan, D.A., Nava, A., Bagattin, A., Devaney, J.M., Stanchi, F., Larderet, G., Brahmbhatt, B., Brown, K., Bauce, B., *et al.* (2001). Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). Hum Mol Genet *10*, 189-194.

Wehrens, X.H., Lehnart, S.E., Huang, F., Vest, J.A., Reiken, S.R., Mohler, P.J., Sun, J., Guatimosim, S., Song, L.S., Rosemblit, N., *et al.* (2003). FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell *113*, 829-840.

Wehrens, X.H., Lehnart, S.E., Reiken, S., van der Nagel, R., Morales, R., Sun, J., Cheng, Z., Deng, S.X., de Windt, L.J., Landry, D.W., *et al.* (2005). Enhancing calstabin binding to ryanodine receptors improves cardiac and skeletal muscle function in heart failure. Proc Natl Acad Sci U S A *102*, 9607-9612.

Wehrens, X.H., Lehnart, S.E., Reiken, S., Vest, J.A., Wronska, A., and Marks, A.R. (2006). Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. Proc Natl Acad Sci U S A *103*, 511-518.

Yano, M., Ono, K., Ohkusa, T., Suetsugu, M., Kohno, M., Hisaoka, T., Kobayashi, S., Hisamatsu, Y., Yamamoto, T., Noguchi, N., *et al.* (2000). Altered stoichiometry of FKBP12.6 versus ryanodine receptor as a cause of abnormal Ca(2+) leak through ryanodine receptor in heart failure. Circulation *102*, 2131-2136.

CLAIMS

What is claimed is:

1. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a compound of Formula I, or enantiomers, diastereomers, tautomers, pharmaceutically acceptable salts, hydrates, solvates, complexes, metabolites, or prodrugs thereof, or any combination thereof.

2. The method of claim 1, wherein the compound is selected from the group consisting of S1, S2, S3, S4, S5, S6, S7, S9, S11, S12, S13, S14, S19, S20, S22, S23, S24, S25, S26, S27, S36, S37, S38, S40, S43, S44, S45, S46, S47, S48, S49, S50, S51, S52, S53, S54, S55, S56, S57, S58, S59, S60, S61, S62, S63, S64, S66, S67, S68, S69, S70, S71, S72, S73, S74, S75, S76, S77, S78, S79, S80, S81, S82, S83, S84, S85, S86, S87, S88, S89, S90, S91, S92, S93, S94, S95, S96, S97, S98, S99, S100, S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123.

3. The method of claim 1, wherein the compound is selected from the group consisting of S36, S47, S50, S64, S74, S75, S77, S101, S102, S103, S107, S110, S111, and S117.

4. The method of claim 1, wherein the compound is selected from the group consisting of S101, S102, S103, S104, S105, S107, S108, S109, S110, S111, S112, S113, S114, S115, S116, S117, S118, S119, S120, S121, S122, and S123.

5. The method of claim 1, wherein the compound is selected from the group consisting of S101, S102, S103, S107, S110, S111, and S117.

6. The method of claim 1, wherein the compound is S36.

7. The method of claim 1, wherein the compound is S64.

8. The method of claim 1, wherein the subject is a mammal selected from the group consisting of primates, rodents, ovine species, bovine species, porcine species, equine species, feline species and canine species.

9. The method of claim 1, wherein the subject is a human.

10. The method of claim 1, wherein the subject is suffering from hemodynamic overload.

11. The method of claim 1, wherein the subject is suffering from hypertension, aortic stenosis, myocardial infarction, congestive heart failure, assymetric septal hypertrophy (ASH), left ventricular outflow tract obstruction (LVOTO), distal cavity oblitaration (DCO), idiopathic hypertrophic subaortic stenosis (IHSS), hypertrophic obstructive cardiomyopathy (HOCM), apical hypertrophic cardiomyopathy, non-obstructive hypertrophic cardiomyopathy, chronic hemodynamic overload, a cardiac arrhythmia or sudden cardiac death.

12. The method of claim 11, wherein the cardiac arrhythmia is selected from the group consisting of ventricular fibrillation, ventricular tachycardia, bradycardia, long QT syndrome, QT 455ms, and an exercise induced arrhythmia.

13. The method of claim 1, wherein the subject has a mutation associated with development of cardiac hypertrophy.

14. The method of claim 13, wherein the mutation is an inherited mutation.

15. The method of claim 13, wherein the mutation is a sporadic mutation.

16. The method of claim 13, wherein the mutation is in the gene that encodes the RyR2 protein.

17. The method of claim 16, wherein the mutation results in increased open probability of the RyR2 channel.

18. The method of claim 16, wherein the mutation results in increased Ca2+ current through the RyR2 channel.

19. The method of claim 16, wherein the mutation results in calcium leak through the RyR2 channel.

20. The method of claim 16, wherein the mutation decreases the affinity with which calstabin 2 binds to the RyR2 protein.

21. The method of claim 16, wherein the mutation increases dissociation of calstabin 2 from the RyR2 protein.

22. The method of claim 16, wherein the mutation decreases binding of calstabin 2 to the RyR2 protein.

23. The method of claim 16, wherein the mutation is selected from the group consisting of an R929C mutation, a G2367R mutation, an R2642K mutation, and a E3654D mutation.

24. The method of claim 1, wherein the compound is administered by a route selected from the group consisting of parenteral, enteral, intravenous, intraarterial, intracardiac, intra intrapericardial, intraosseal, intracutaneous, subcutaneous, intradermal, subdermal, transdermal, intrathecal, intramuscular, intraperitoneal, intrasternal, parenchymatous, oral, sublingual, buccal, rectal, vaginal, inhalational, and intranasal.

25. The method of claim 1, wherein the compound is administered using a drug-releasing implant.

26. The method of claim 1, wherein the compound of Formula I is administered to the subject at a dose sufficient to restore binding of calstabin 2 to RyR2.

27. The method of claim 1, wherein the compound of Formula I is administered to the subject at a dose sufficient to enhance binding of calstabin 2 to RyR2.

28. The method of claim 1, wherein the compound of Formula I is administered to the subject at a dose of from about 0.01 mg/kg/day to about 20 mg/kg/day.

29. The method of claim 1, wherein the compound of Formula I is administered to the subject at a dose of from about 0.05 mg/kg/day to about 1 mg/kg/day.

30. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases the open probability of the RyR2 channel.

31. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases Ca2+ current through the RyR2 channel.

32. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases calcium leak through the RyR2 channel.

33. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases the affinity with which calstabin 2 binds to RyR2.

34. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that decreases dissociation of calstabin 2 from from RyR2.

35. A method of treating cardiac hypertrophy in a subject in need thereof, comprising administering to the subject a therapeutically or prophylactically effective amount of a compound that increases binding of calstabin 2 to RyR2.

Figure 1

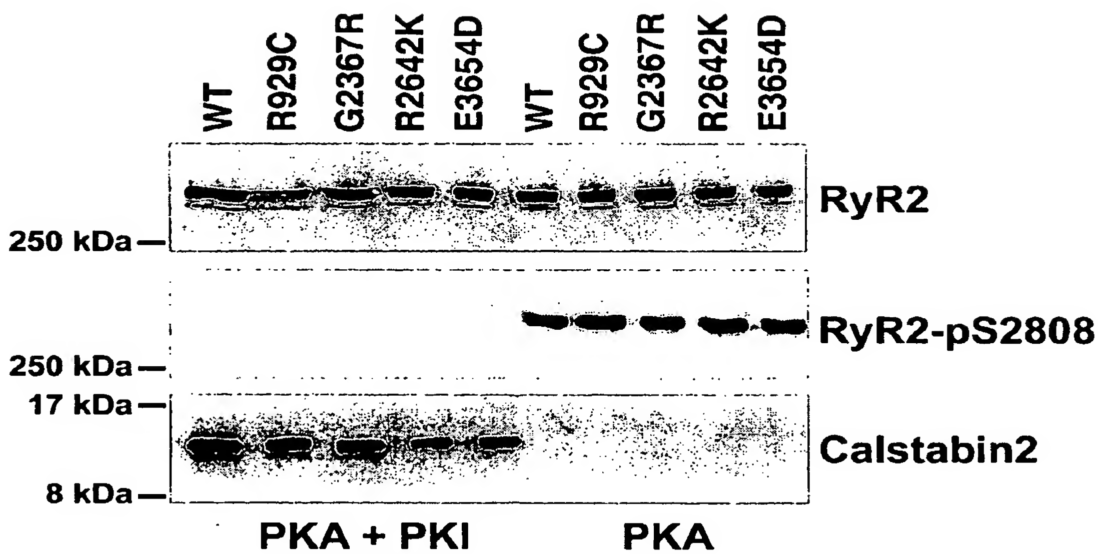
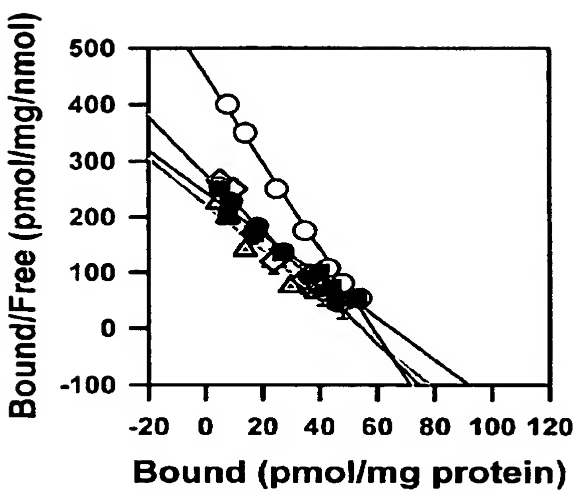

WT
R929C
G2367R
R2642K
E3654D
WT
R929C
G2367R
R2642K
E3654D
RyR2
250 kDa
RyR2-pS2808
250 kDa
17 kDa
Calstabin2
8 kDa
PKA + PKI
PKA

Figure 2



	K_d (nM)	B_{max} (pmol/mg-μM)
○ RyR2	128	58
● RyR2-R929C	235	64
■ RyR2-G2367R	266	65
△ RyR2-R2642K	175	56
◇ RyR2-E3654D	200	55

Figure 3

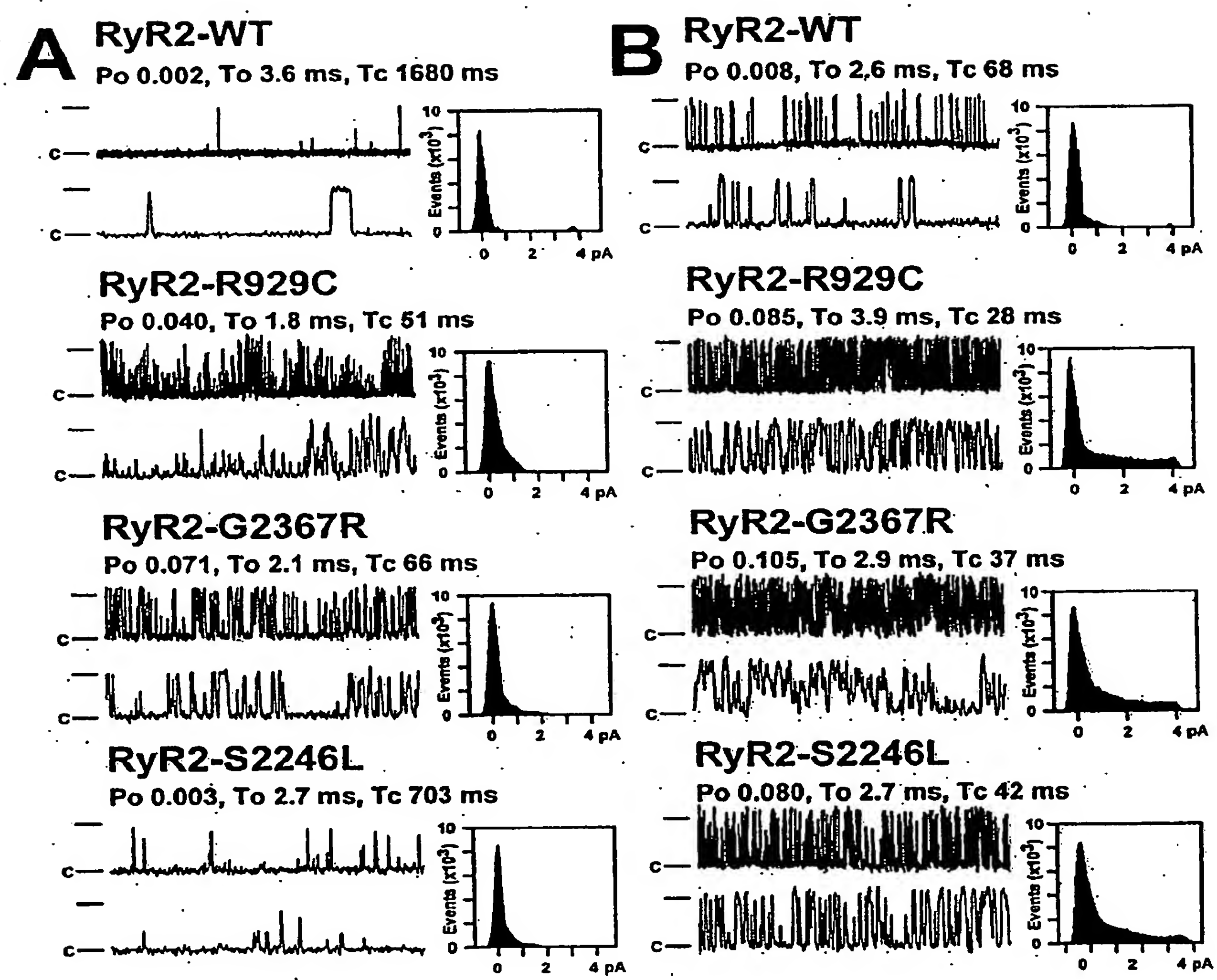
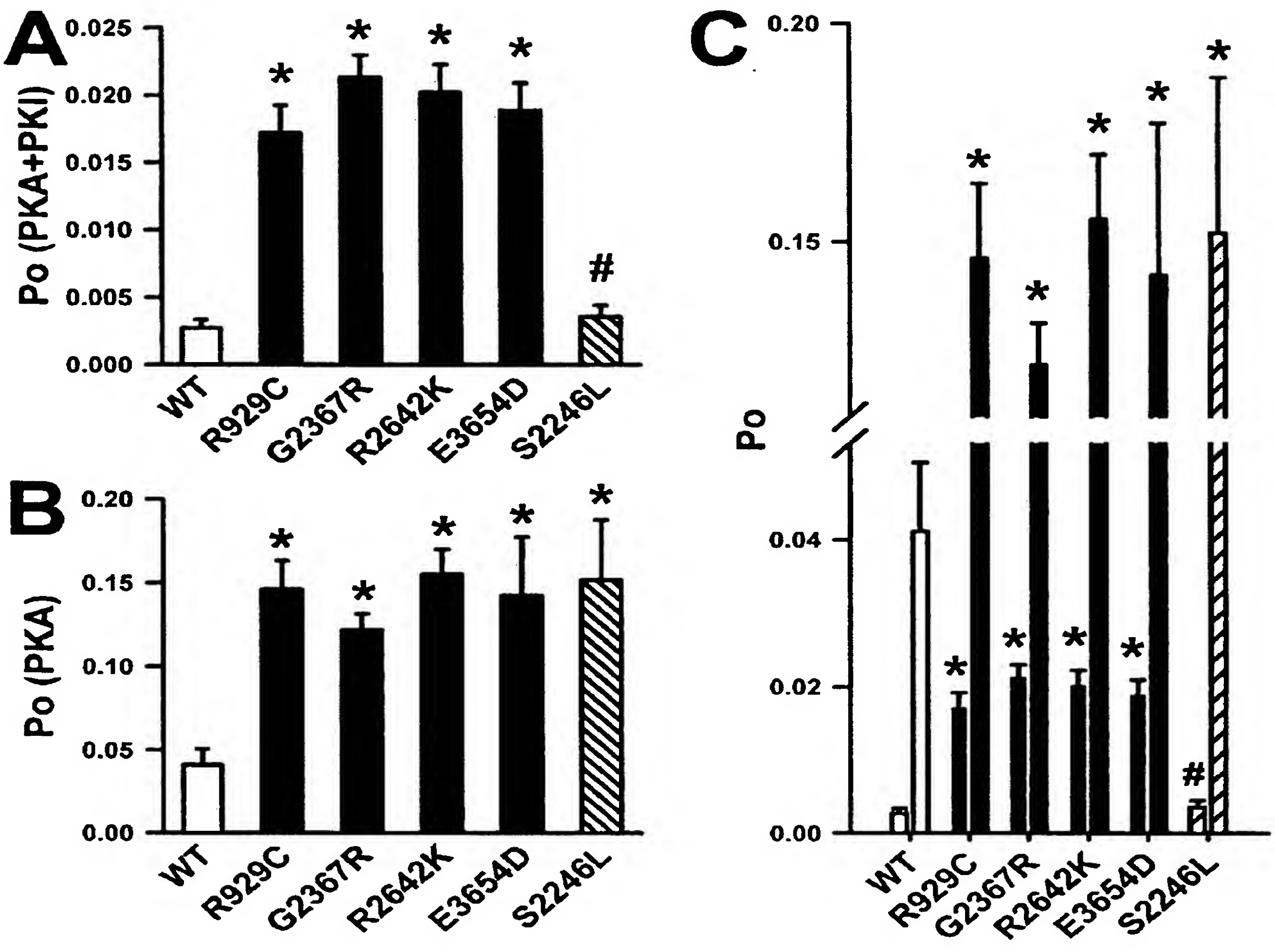

A
RyR2-WT
Po 0.002, To 3.6 ms, Tc 1680 ms
RyR2-R929C
Po 0.040, To 1.8 ms, Tc 51 ms
RyR2-G2367R
Po 0.071, To 2.1 ms, Tc 66 ms
RyR2-S2246L
Po 0.003, To 2.7 ms, Tc 703 ms
B
RyR2-WT
Po 0.008, To 2.6 ms, Tc 68 ms
RyR2-R929C
Po 0.085, To 3.9 ms, Tc 28 ms
RyR2-G2367R
Po 0.105, To 2.9 ms, Tc 37 ms
RyR2-S2246L
Po 0.080, To 2.7 ms, Tc 42 ms
c
Events (x10³)
0
2
4 pA
10

Figure 4


A
Po (PKA+PKI)
0.025
0.020
0.015
0.010
0.005
0.000
WT
R929C
G2367R
R2642K
E3654D
S2246L
B
Po (PKA)
0.20
0.15
0.10
0.05
0.00
C
Po
0.20
0.15
0.04
0.02
0.00